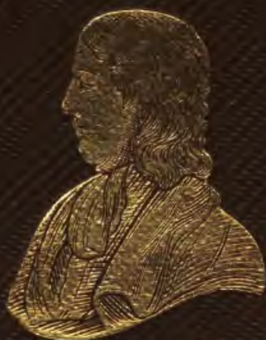

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THE FUNDAMENTAL DATA

OF

MODERN PATHOLOGY

HISTORY, CRITICISMS, COMPARISONS,
APPLICATIONS.

BY

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TRANSLATOR'S NOTE.

"Novi veteribus non sunt opponendi, sed quoad fieri potest perpetuo jungendi fœdere."
BAGLIVI, *De praxi medica.*

THIS work was awarded the prize at the Fondazione Cagnola Competition, held in 1897, on the following theme proposed by the Royal Lombard Institute: "What influence has the doctrine of proliferation of the cells beyond the normal exercised on the pathology of man? What has that of the pathogenic microbes? Contrast the two doctrines with others older. The advantages of both in the treatment of human infirmities."

Inasmuch as the manuscript was presented anonymously to the competition signed only with the above motto, Professor Monti, in order to maintain his incognito in the bibliographical references as well as in the text, was obliged to quote his own previous writings in the third person. In the following year (1898) the work was published at Turin, the author having made some additions to the manuscript so as to bring it up to date. For this translation Professor Monti has been good enough to write, at my suggestion, several supplementary pages on the origin of tumours, and on the recent very important and interesting discoveries in relation to malaria, which I have embodied in the respective articles.

JOHN J. EYRE.

31, PIAZZA DI SPAGNA, ROME;
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INTRODUCTION.

"THERE is and ought to be a militant, explanatory, and experimental science which I would call the vanguard of science; but it is also necessary that the body of consolidated science should not exceed the strength of the army which examines and tests the things discovered, compares them with those already known, and estimates them for what they are worth. Without this prudent procedure there would be a chaotic science."

To this judgment, expressed by Sangalli more than twelve years ago in a work on 'Cells and Parasites in Pathology,' we can also to-day fully subscribe.

Such judgment shows the necessity, now felt by many, of subjecting to critical and synthetic revision the innumerable congeries of new observations and new doctrines that in this century have accumulated in science. And such must have been the thought of the *Istituto Lombardo* in proposing the thesis which we intend to develop.

In the course of our work we shall endeavour to recognise the historical rights and the merits of our great predecessors; since nowadays hearing those who consider their own little discoveries great ones, with much levity speak disparagingly of the work of the old masters, makes in reality a disagreeable impression. It is very true that one ought to defend

his own rights, and that one does not practise science solely for the purpose of adding new cognitions to the old, but also to make them known and appreciated by others. This appreciation chiefly rests upon the faith which others have in our investigations, on the consideration they accord to our work. It equally behoves us, then, not to forget the work of others, and especially that of our predecessors; we must co-ordinate our work with that of the past generations, assigning them their due merit in relation to the development of medical science.

In our analysis of the older theories we shall find in general many indistinct notions, derived not from direct observation and positive induction, but only from the vague intuitions of physio-pathological laws. The patient work of modern science has given a positive basis to many hypothetical theories of the ancients, and has founded the laws of diseased life on minute researches rigorously tested and re-tested. It, at the same time, has demonstrated that many of the ideas of the older writers were erroneous; but even of these, as Frey has written, we must not judge with excessive severity, because frequently error, pushing along science in its path of research, and summoning it to control hypotheses, has also served to discover truth.

The fundamental discoveries which have given a special stamp to the pathology of the nineteenth century, and have brought it into a prolific field of useful results, certainly not foreseen before our epoch, are those which constitute the basis of the "cellular pathology" and of the "parasitic pathology," which integrating have solved the problem propounded by our great Morgagni, that is to say, the demonstration of the seats and of the causes of diseases.

Cellular pathology was able to develop when it was demonstrated that the organism of every higher animal is a federation of elementary organisms, endowed with fundamental biological activities; it was the demonstration of the proliferation of the cells in the normal and pathological tissues (*omnis cellula e cellulâ*) which has given the greatest impulse to this doctrine.

Parasitic pathology has originated the positive study of the causes of the contagious and epidemic diseases,

especially by the demonstration of the bacterial parasites and of their pathogenic action on animals.

We, therefore, shall analyse in separate chapters the doctrine of the proliferation of cells and the general doctrine of pathogenic microbes, demonstrating what influence both doctrines have exercised on pathology. In other chapters we shall compare these doctrines with others older, endeavouring to determine the development of the cognitions now universally accepted; in fine, we shall briefly summarise their most important applications.

CHAPTER I.

THE DOCTRINE OF CELLULAR PROLIFERATION; ITS INFLUENCE ON THE PATHOLOGY OF MAN.

THE doctrine of the proliferation of the cells beyond the normal has its foundation in the numerous studies of histology, a new science which has arisen in the nineteenth century.

He who wishes to analyse the influence that this doctrine has exercised on the pathology of man must set out from the fundamental studies on the development and the growth of the normal organism, considering the laws of such biological phenomena, and then searching in what way these laws act in the diseased organism.

Thus, to be able to determine what importance the doctrine of the proliferation of cells has, it will be necessary, starting from general histology, to pass in review the whole of cellular pathology.

And this is required also by the essence, the nature, and the conclusions of this doctrine, which applies to the diseased organism the same fundamental laws that govern the life of the elements in the healthy organism.

Such doctrine arises from the objective study of the pathological processes, and from the minute analysis of their development in the respective primary seat. It is through this a result of the positive method of scientific investigation, a method which the pathologists, misled by philosophic preconceits, had too long forgotten.

Morgagni[1] in the last century, in his immortal book on the 'Seats and Causes of Diseases,' had attempted to confine

[1] Morgagni, 'De sedibus et de causis morborum per anatomen indagatis,' libri quinque, Venet., 1761, f. 2.

medicine to its place of investigator of matter, designating to it the task of studying the alterations of the organs, and how these alterations cause the various manifestations that characterise disease clinically studied. But minds were then too much imbued with the metaphysical ideas on life for the seed scattered by Morgagni to find a soil suitable for its growth. They continued to build pathology on the sketches of Stahl and Brown, instead of studying it in the cadaver and at the bedside of the patient.

In other countries, on the contrary, and especially in Germany, where the natural sciences and natural philosophy had largely diffused the ferment of research, Morgagni's idea, accepted and developed by powerful minds, became the point of departure of prolific analytical work, owing to which medicine was finally able to reach the dignity of a positive science.[1]

Thus it came to pass that the medicine which followed the guidance of Morgagni is called German, and the medicine Italian that followed the vitalistic ideas of Stahl and Brown. The doctrine of which we shall now treat arose, indeed, in Germany, but it cannot be erased from history that it, in its method and direction, had its origin in Italy, through the work of our illustrious Morgagni.

Remarks on the Cellular Pathology.

The doctrine of the pathological proliferation of cells is founded upon the cellular theory of life and of disease.

In 1842 Schwann had generalised the cellular theory of the organism; he demonstrated that all the superior beings are an aggregate of cells,—that is to say, of small elements which feed, grow, and multiply; they possess self-movement, secreting particular substances,—that is, they present all the activities proper of living beings. The organism, consequently, ceased to be an indivisible unity, as it was for the old vitalists; it, according to the able expression of Brücke, is an aggregate of elementary organisms.

[1] Cf. Golgi, 'Lo sperimentalismo nella medicina,' Pavia, 1883; cf. Virchow, 'Morgagni und des anatomische Gedanke,' XI Congr. medico-internaz., Roma, 1894, vol. i.

To these elements of our organism it is necessary to recur, Claud Bernard has well said, if one wishes to understand the mechanism of all the physiological and pathological actions. Histology, therefore, raised to the position of an autonomous science, especially through the work of Remak and of Kölliker, thus became the basis of physiology.

The cellular theory of life gradually led to the cellular theory of disease. In fact, after the manifold tentatives of various authors (see the chapter where this doctrine is contrasted with other older doctrines), Virchow, in the year 1858, published his memorable book on Cellular Pathology.[1] Once demonstrated that physiological life results from the regular functioning of cells, and in consequence from their normal constitution, their perturbations must give rise to disease, and manifest themselves by external signs.

The necessity then was felt of searching for the alterations of the cells by means of powerful microscopes and with the aid of diverse methods, which the allied sciences furnished to pathology.

This research led the pathologists away from the abstract and aphoristic lucubrations of vitalism and of morbid principles, to the objective consideration of the pathological alterations of the individual cells.

It was Virchow who, systematising the multiple labours of his predecessors, seized that historical moment in the development of the science, and initiated a new era in pathology.[2]

Before Virchow, pathology in general, whether humoral or solidist, admitted morbid efficiencies in act, the greater part of which were still believed diathetic or general; but it did not know, or it imagined without searching, where these general *quid* were situated, and when it accorded them a circumscribed seat it was ignorant of the organic elements affected in disease. Some pathologists, in fact, founded local diseases on innervation, others on the blood of the organ or on the vessels, with or without transudation, and thus hyperæmia and arteritis were the obligatory exponents of almost all local diseases.

[1] Virchow, 'Die Cellularpathologie,' Berlin, 1858.

[2] Cf. Salvator Tommassi, 'La Riforma degli studî Medici in Italia,' Napoli, 1878.

The dyscrasic element was also admitted by some, but no enlightenment subsequently arose from it, and thus the doctrinal exposition was so vague and indetermined that it escaped from the most keen criticism.[1]

In this state of pathology Virchow appeared, who, guided by histology and embryology, wrote, "We find ourselves in the midst of a great reform in medicine.

"For the first time all the vast dominion of this science is subjected to positive investigation. The precepts, which date from immemorial time, are submitted to the test of observation and to that of experiment. By observation is obtained the verification of facts; by experiment the precise methods for re-proving them.

"Above all, observation is directed on the ultimate elements accessible to the senses of man; medical science is divided into an enormous heap of individual facts, which appear to confuse the mind of many.

"But for him who masters the facts on which the new views are founded, an order is revealed, that, departing from a general biological principle, permits the new discoveries to be connected according to their value, according to their mutual relations—into a scientific whole.

"This order is the cellular doctrine, which, applied to all living bodies, leads to a cellular physiology and to a cellular pathology, both of which are founded upon the anatomical knowledge of the elements,—that is to say, upon histology."

Schwann had already popularised the idea that only one solitary elementary form, the cell, passes through the whole organic world always remaining constant; he had already demonstrated that it was useless to try to substitute any other element whatever for the cell.

Virchow was thus led to consider the higher organisms as the resultant of a variable number of cells, which contain, each one in itself, all the attributes of life. The higher organism, then, is the sum of numerous living units; it is the result of a social organisation of many elements,—that is, it is constituted of a mass of individual existences, which, uniting together, divide the work and are perfected. But

[1] Cf. Tommassi, l. c., and Monti, 'La patologia cellulare e la patologia parassitaria,' Milano, Vallardi, 1890.

this union is such that it does not interfere with the particular activities of each element, so that even when other parts impress an impulse on it, the function which it fulfils always belongs to the element itself.

The pathological elements have nothing specific: they find a perfect counterpart in the normal tissues pre-existing in the economy. All the cells are derived from other cells.

In pathology, as in physiology, there are no new creations: this law is as true for the elementary organisms as it is for the complex organisms. Just as worms do not originate from putrefaction, but are generated by other similar beings, so also in physiological and pathological histology there is no possibility of a cellular formation by an amorphous substance: *omnis cellula e cellulâ.*[1] There is no discontinuity of development between the normal and pathological elements, just as there is no spontaneous generation of simple vegetable or animal organisms.

All the normal and pathological tissues already developed can be reduced to a simple element, and this is the cell, whose prototype is the ovum.

To explain the obscure origin of many pathological tissues Virchow developed the knowledge of the connective tissue, and demonstrated that it presents cells capable of multiplying themselves, and of giving rise to abnormal productions under the influence of morbid excitations.

When studying the alterations of the blood, Virchow recognised that the dyscrasias are due to the introduction into the blood of noxious substances, and he invited the studious to search for the altered cellular groups which cause the blood to undergo ulterior alterations. Examining and studying the hæmatopoietic organs in a methodical way, he completed his remarkable researches on leukæmia and leucocytosis; analysing pyæmia, he explained for the first time its mechanism, demonstrating the part which embolism and thrombosis take in it.

Disease in act consists of an altered cellular activity, awakened by a chemical, physical, or mechanical stimulus; by such disorder the metabolism of the cells usually becomes

[1] Virchow, "Cellularpathologie," 'Virchow's Archiv,' vol. viii, fasc. 1, 1855.

tumultuous, and the cellular contents consequently remain altered. Or it may happen that the stimulated cells undergo degeneration, through which the function of the organ becomes impeded; or, finally, it may also happen that the cells, owing to the irritation, proliferate, and thus lead to the histological formation of pathological tissues.

All these doctrines, developed by Virchow with the accompaniment of a great number of anatomical facts, chiefly discovered by himself, exposed with clear and concise language, sometimes bellicose and biting, convinced the majority of the studious of that epoch.

Never before then was the attempt made to follow the normal and pathological functions of the organism to its most minute elements, and never before had the knowledge of the physiological and pathological processes made such great progress as after Virchow had founded the cellular pathology.

"I express the hope," said Virchow, "that the pathologists, renouncing the metaphysical systems, will recognise that only indefatigable labour, the faithful work of observation and experiment, has a lasting value. The physiology of diseased man will then gradually develop, not as the creation of a few able minds, but as the result of the researches of many patient observers.

"Many studies will still be necessary, because science is not built with the simple generalisation of some isolated facts.

"We have completed only the first part of the work; the task of continuing it rests with other hands."

In reality, Virchow's book on Cellular Pathology mapped out a complete guide to scientific medicine, and to finish the work nothing remained to his successors but to follow the same path into which Virchow so resolutely entered.

The synthesis of Virchow, however, like all works that tend to reunite multiple scattered observations into a body of doctrine, could not necessarily solve in a moment all the problems of pathology. It is very natural, on the contrary, that the cellular pathology, owing to the rapidity itself with which it was constituted, could not immediately disclose all the genuine characteristics of the individual morbid processes; and, indeed, we find that Virchow himself has not been perfectly exact in his generalisations; but his doctrine as a

whole had an absolutely stabile basis of facts. Many new chapters have been added to it; many obscure points have been cleared up; in the interpretation of many phenomena various views have been more or less radically changed; but it has always been demonstrated—and every new progress in the science has given ulterior and more valid proofs of it—that morbid processes have their basis in the alterations of the activities and of the life of the cells, which are autonomous elements comparable to the lower organisms.

The Proliferation of Cells.

The doctrine of pathological proliferation formulated by Virchow has undergone many notable modifications in its particulars, but it remains fully confirmed in its general principles.

The *fact* of the multiplication by scission in the normal cells was observed for the first time by Mauro Rusconi [1] when he, in 1826, discovered the segmentation of the ovum of the frog. But the *theory* of the proliferation of cells was formulated by Remak and by Koelliker only after Schwann had founded the cellular doctrine. Virchow, following the lines of Remak and partly adopting the ideas of other pathologists who had preceded him, admitted two principal modes of cellular proliferation,—that is to say, proliferation by fissiparity and that by endogenesis.

The reproduction by fissiparity, also called by fission, represents the commonest mode of proliferation of the cells; endogenesis, on the contrary, is, to a certain extent, the exception.

As regards the mode in which scission occurs, Virchow wholly adopted, for the pathological tissues, the schema described by Remak. That is, he maintained that, following the formative irritation (whose nature it is not now necessary to discuss), the first phenomenon which is verified is the extraordinary enlargement of the nucleolus, which in many cases elongates somewhat, at times in the form of a rodlet. Then follows as its next stage the indentation of the nucleolus itself in its median part, so that it takes the shape of a disc. Later,

[1] M. Rusconi, 'Développement de la grenouille commune depuis le moment de sa naissance jusqu'à son état parfait,' Milano, Giusti, 1826.

the indentation continuously deepening, the nucleolus divides into two. The bipartition of the nucleolus indicates the imminent division of the nucleus, which in its turn elongates, takes the form of a bisac, is constricted like the figure of 8 into two halves, each of which contains a nucleolus, and finally divides into two, while in its turn the protoplasmic body is constricted, and divides into two equal parts, each containing a nucleus.

This mode of cell division still passes under the name of *direct division according to Remak's schema*.

However, it is necessary to mention that Virchow himself was the first who, in 1857 studying the pathological proliferation in the cells of a carcinoma,[1] described nuclei indented with multiple incisions, so that they presented a stellate form. It is evident, therefore, that Virchow had seen, at least in great part, the most minute phenomena of nucleus division, which later on was described with the name of *karyokinesis*. We must also mention that Remak,[2] in 1858, represented the blood-corpuscles of the embryo of the fowl undergoing division, in which the nuclei appeared formed by threads arranged longitudinally. Flemming, therefore, rightly considers Virchow and Remak as the discoverers of some karyokinetic forms.

The second mode of cell proliferation admitted by Virchow was called *by endogenesis* by him. Virchow stated in a precise way that within a simple cell, especially in cancerous tumours, but also in normal tissues, as the thymus, particular spaces, vesicles, or physales are formed, that Virchow also called hatching spaces or generating spaces. Within these spaces new cells gradually develop, very probably following the division of the nucleus of the mother cell.

Besides Virchow, Remak,[3] Buhl,[4] and Rindfleisch[5] have

[1] Virchow, "Ueber die Theilung der Zellenkerne," 'Virchow's Archiv,' vol. xi, fasc. 1, 1857, p. 89.

[2] Remak, "Ueber die Theilung der Blutzellen bei Embryo," 'Müller's Archiv,' 1858, p. 178.

[3] Remak, "Ueber endogene Entstehung von Eiter- und Schleimzellen," 'Virchow's Archiv,' vol. xx, p. 198.

Buhl, "Ueber die Bildung der Eiterkörperchen," 'Virchow's Archiv,' vol. xvi, p. 168, 1859, and 'Sitzungsberichten der Münchener Akad.' 1863.

[5] Rindfleisch, 'Pathologische Histologie;' see also "Ueber die Entste-

described the formation of mucus and pus cells within epithelial cells. In some cases it was not all the mother cell that was transformed into new cells, but only a part of its contents, and, according to some, after the division of the nucleus.

This mode of proliferation, also observed by Rokitansky, was, however, not accepted by more recent authors. In fact, Steudener,[1] like Volkmann, had asked himself if a cell completely enveloped by the flexible body of a neighbouring cell would not present the image of a daughter cell contained in the body of a mother cell.

Bizzozero [2] in a notable work solved the question of endogenesis in the negative sense. He studied the pus that formed in the anterior chamber of the eye, where numerous large cells containing pus corpuscles were frequently found. After a series of observations made on man, and numerous experiments on animals, he came to the conclusion that the cells contained in the protoplasm of other cells are not really due to a process of endogenesis, but to the penetration of pus cells into some of the large cells, which gradually develop with the growing old of the pus. The penetration of the small cells into the large ones takes place owing to the contractility of the latter. He saw these large amœboid cells ingest not only small lymph-cells, but also red blood-corpuscles. To prevent some believing in an endogenous production of red corpuscles, Bizzozero injected into the anterior chamber red corpuscles different from those of the experimental animals (for example, red corpuscles of the fowl into the anterior chamber of the eye of a rabbit), and he still saw the large elements ingest the red corpuscles which had been introduced. Bizzozero observed, besides, that the pus corpuscles enclosed in the large cells have all the characters of old cells undergoing disintegration ; he noted also that the cells containing other cells were not met with in the period

hung des Eiters auf Schleimhäuten," 'Virchow's Archiv,' 1861, vol. xxi, p. 486.

[1] Steudener, "Ueber invaginirte Zellen," 'Arch. für mikroscopische Anatomie,' vol. iv, p. 188.

[2] Bizzozero, "Sulla endogenesi del pus," 'Gazzetta med. lombarda,' Milano, 1872.

of formation of the pus, but made their appearance some time after the latter had gathered, and when a number of its leucocytes were already dead.

To sum up, Bizzozero came to the conclusion that an endogenous production does not take place, but an ingestion of corpuscles into the contractile protoplasm of the large cells of connective-tissue origin.

We have somewhat lengthily referred to these studies of Bizzozero inasmuch as they have fundamental importance in destroying the theory of pathological endogenesis; they represent, besides, a new discovery, that of *phagocytosis*, which much later on, accepted and defended by Metschnikoff, acquired so much importance in parasitic pathology.

The very accurate successive observations on the histology of neoplasms, made by very many authors, have generalised the ideas of Steudener and Bizzozero, and have demonstrated how other elements penetrate into the epithelial cells by invagination, and thus they have disproved the theory of endogenesis.

Scission, therefore, remained as the only method of cell multiplication—the general foundation not only of the development of the normal tissues, but also of the pathological proliferations.

The process of scission was not only generally confirmed, but also so studied in its most minute particulars that it led to the establishing of laws much more precise and rigorous than those of the simple schema of Remak.

In fact, the researches instituted at first on plants and the lower animals have demonstrated the intimate structure of the nucleus, and the special changes that such structure undergoes when the process of cell multiplication begins.

It is not necessary to trace here the whole history of these new studies, which have led to the discovery of exact laws that indicate precisely which are the proliferating elements, which, on the contrary, are the cells at rest; and also distinctly characterise the various phases of the process of scission. In connection with the observed particularities, and especially with the minute phenomena that are observed in

the nucleus, the name of *karyokinesis*, proposed by the zoologist Schleicher,[1] has been given to the *ensemble* of the process.

We have mentioned already that Flemming, one of the discoverers of karyokinesis, has credited Virchow and Remak with the first observations on the argument; Mark refers it back still farther, and records how Grube (1844), Derbes (1847), Reichert (1847), De Quatrefages (1848), Kron (1852), Meisner (1854), and Gegenbaum (1857) had already observed the *Aster* or *Amphiaster* forms, or, in whatever mode, some of the modifications of the nucleus or of the protoplasm in the ova or the spermatozoa of the lower animals.

Mayzel[2] was, perhaps, the first who described in a complete way the process of indirect scission or *karyokinesis*.

But the importance of this process was not fully estimated till after the memorable labours of Flemming.[3] Towards the end of the year 1879 the indirect division of the cell, or karyokinesis, was a fact already definitely acquired by science, and considered as a normal phenomenon verified in the animal as well as in the vegetable kingdoms (Strassburger). The subsequent observations of innumerable authors demonstrated that this mode of cell multiplication is observed in all the elements of the various tissues; they established that the process of division is everywhere equal in its essence, and that the various forms of division which have been described could be included in a general schema.

The general schema of karyokinesis may be summarised as follows.

The completely developed nucleus of a cell presents a particular structure, which can be recognised by means of suitable stainings and with high power objectives (homogeneous immersion). A resting nucleus consists of a very thin membrane and of contents divisible into a nuclear substance and a nuclear juice. The nuclear substance

[1] Schleicher, "Ueber den Theilungsprocess der Knorpelzellen," 'Centralbl. f. medicinische Wissenschaften,' 1878, p. 418.

[2] Mayzel, "Ueber eigenthümliche Vorgänge bei der Theilung der Kerne in Epithelzellen," 'Centralblatt f. d. med. Wissenchaften,' 1875, N. 50, p. 849, and 'Gazeta lekanska,' N. 26, 1876.

[3] Flemming, 'Zellsubstanz, Kern und Zelltheilung,' Leipzig, Vogel, 1882.

consists of nucleoli, and of granules and filaments that frequently form a *reticulum*.

The nuclear reticulum is that part of the nucleus which during the process of proliferation undergoes a series of typical changes of form, whose final result is the division of the nucleus. The principal constitutive substance of the nuclear reticulum was called *chromatin* by Flemming, because it becomes stained with particular reagents; it has also been studied chemically by Miescher, Kossel, and others, who gave it the name of *nuclein*.

If we follow the process of proliferation in the tissues of man or of the mammifers, we see that the division of the nucleus is preceded by a series of structural modifications, which consist principally in an increase of the chromatin. In many nuclei this substance forms nodules of various sizes united by thin threads disposed in a network; in others, on the contrary, uniform granules form round the nucleolus, or chains of granules more or less bent upon themselves are formed.

In a successive phase the granules and threads of the reticulum are condensed into thicker, smooth, and intensely stainable filaments, which form a ravelled skein; thus one has the stage of *skein* or *spirem*. At this point the nucleolus and the nuclear membrane disappear together.

Later the filaments become shorter, thicker, more distinct from one another, and are separated into segments, which have a fixed number for each species of cell, and which Waldeyer called *chromosomes*. The name of *segmented skein* has been given to this nuclear movement.

The chromosomes then move towards the equator of the cell, and are grouped at a right angle round the axis of the cell, in such a way that when the cell is observed from a pole the nucleus presents the form of a star, called the *mother star* or *aster*.

Already, in a period preceding the formation of the mother asters, notable modifications have also taken place in the protoplasm. The *centrosome*, a protoplasmic body that may frequently be observed near the nucleus even in resting cells, divides into two, forming the *polar corpuscles*, round which the granulations of the protoplasm collect in a radial disposi-

tion. The two corpuscles separate and migrate towards the poles, remaining united together by the so-called *nuclear spindles.*

In a successive phase, called metakinesis, the chromosomes split longitudinally, and one half of them move towards one pole and the other half towards the other.

Thus by degrees two asters, which are called *daughter asters*, are formed round the two corpuscles; the corresponding stage is called *diaster*.

If the cells that are found in this phase are observed from one side, the two asters, especially if they are still near each other, cannot be clearly distinguished, and they form a figure which has been compared to that of a barrel.

The chromosomes of the daughter asters then agglomerate and form two *daughter skeins*—the stage of *dispirem.* The daughter skeins in their turn become compact, their threads become thinner and nodular, and are transformed into two reticula with nucleoli.

At the beginning of the *amphiaster* phase the protoplasm also passes round the two polar globules, withdrawing from the median part, so that at the end of the nucleus division the complete division of the protoplasm has also occurred.

This mode of cell multiplication was very quickly observed also in numerous pathological tissues by very many authors, such as Bizzozero, Golgi and his pupils, Krafft, Pfitzner, Podwyssoski, Arnold, Kosinscki, Martin, Cornil, Seslawin, Blonski, Eberth, Hansemann, etc.

Strassburger[1] afterwards, studying the hairs of the *Tradescantia virginia,* and other vegetable tissues, was able to follow karyokinesis step by step in vegetable tissues to the division of the cell into living tissue. Thus it has been indisputably proved that the above-described typical modifications of the nucleus truly express a process of cell division.

Since the proof given by Strassburger, and subsequently confirmed by many others, also by observations made in ova, Protozoa, etc., it has been generally admitted that karyo-

[1] Strassburger, 'Zellbildung und Zelltheilung,' 3 Aufl., 1880, and 'Histologische Beiträge ueber Kern und Zelltheilung in Pflanzenreiche, etc.,' Jena, 1888.

kinesis does not represent a more or less probable theoretical construction, but a concrete fact, recognisable at will in living beings.

The observation of cell division in the living tissues has also permitted the time to be determined which is necessary for a cell to divide into two. Nowadays it has been demonstrated that the entire process is completed within three hours, and for some elements even in a shorter time. Flemming, for example, has seen divisions completed in the brief space of half an hour.

Already the first authors who have occupied themselves with karyokinesis in the normal and pathological human tissues immediately recognised the light that the new discovery has thrown on pathology.

Such advantages may be briefly summarised in four categories; first of all, the demonstration of the karyokinetic figures gives us the proof of the proliferation of the elements of the tissue itself, and furnishes a very exact index of it for judging the more or less great proliferating activity of that tissue in those given conditions. Before the phenomena of karyokinesis were known the proliferating activity of the cells was recognised only by the observation of some figure-of-8 nuclei, not easily recognisable in the midst of the other elements. The difficulty of the research had much discouraged many observers, and maintained the doubts of those who still supported the doctrine of free formation.

The numerical relations of the karyokinetic figures observed in various periods permit us to measure the intensity of the proliferation, and to establish the laws with which it occurs in the normal and in the pathological tissues. Thus, for example, Bizzozero[1] has demonstrated that in the normal organism there are determinate laws which regulate the increase of the tissues, and he has established that the completely developed tissues of the adult normal individual can be distinguished into three groups. In the first group are those tissues whose elements continue to multiply during the whole life of the individual, thus producing a continuous

[1] Bizzozero, "Accrescimento e rigenerazione nell' organismo," 'Arch. p. le Scienze mediche,' vol. xviii, N. 3.

renewal. Bizzozero calls these *tissues with labile elements.* They belong to the parenchyma of the glands secreting morphological elements (spleen, bone marrow, lymphatic glands, ovary, testicle), the epithelia of investment (epidermis, intestinal epithelium, etc.), and their glandular depressions, such as the muciparous alveoli of the stomach, the tubular glands of the intestine and of the uterus, the sebaceous glands.

To the second group appertain those tissues whose elements multiply by division up till birth, or even for some time after birth,—that is until these elements have assumed their specific characters, and thus, for example, the non-striated muscle cells have already a spindle shape and the mantle of contractile substance, the connective-tissue cells are already flattened and surrounded by fibrillar substance, and so on.

Having passed these limits, however, the elements no longer multiply, and no process of renewal is noted in the tissue. To this group belong the tissue of the glands with an amorphous secretion (liver, kidney, pancreas, salivary and lachrymal glands, etc.), the connective tissue, cartilaginous tissue, and osseous tissue (in which mitosis is prolonged to the complete development of the skeleton) ; finally, the non-striated muscular tissue. These tissues Bizzozero calls *tissues with stable elements*

In fine, to the third group appertain the striated muscular and the nervous tissues. In them the multiplication by mitosis ceases in a very early period of embryonic life, before the elements have yet assumed their specific characters,—that is before the muscle cell has elaborated the contractile substance, and the nerve-cell has sent off its multiple prolongations. This period closed, in mammifers neither the number of the nerve-cells nor that of the striated muscle-fibres any longer increases. Therefore Bizzozero designates these as *tissues with perennial elements.*

From such observations on cellular proliferation in normal conditions important criteria for pathology are derived; in fact, if in the tissues of the second group we find karyokinetic figures, such figures will express an abnormal condition,—that is to say, a regeneration of tissue following a loss of substance, or a hyperplastic condition, or the beginning

of a neoplasm. In the tissues of the third group the losses of substance will not be followed by complete regeneration of the specific elements, but always by the formation of a connective-tissue cicatrix.

A third category of criteria which karyokinesis offers to pathology are those that permit the judging of the direction of the increase of the tissue from the axis of the proliferating figures.

In the epithelia which clothes a surface in general the axis of the mitoses is parallel to the same surface if the increase takes place in a horizontal direction,—as, for example, in the epithelia of the intestine.

The axis of the divisions is vertical, on the contrary, when the increase occurs in depth,—as, for instance, in the epidermis.

In the pathological proliferations, from the study of the axis of mitoses we obtain an idea of the mode of extension of the neoplasm,—that is to say, whether it tends to spread in one determinate direction or in all directions.

Finally, from the study of karyokinesis in the normal and pathological tissues we begin to have an indication of the diverse functional signification of the individual constitutive elements of the nucleus and of the cell body. Such studies are not yet complete, but they already indicate the possible solution of the difficult and obscure problems in connection with pathological heredity.

Compensatory Hypertrophy.

From what we have already stated, it results that the study of cell division has exercised a profound influence on pathology. It has enabled us to solve many fundamental questions relative to compensatory hypertrophy, to the processes of regeneration of destroyed parts, to inflammation, to hyperplasia, and to morbid-tissue tumours.

With regard to compensatory hypertrophy, and to the part that the proliferation of the cells takes in it, we must, above all, consider the studies of Golgi[1] on compensatory hyper-

[1] Golgi, "Sulla ipertrofia compensatoria dei reni," 'Arch. p. le Scienze med.,' vol. vi, N. 20,

trophy of the kidneys. Golgi has demonstrated that following nephrectomy "the epithelia of the uriniferous canaliculi of the remaining kidney, far from bearing themselves in a passive way, as Tizzoni and Pisenti thought, take, on the contrary, a very active part, and they show an accentuated process of proliferation. Nor is there a stimulation of the formative activity only in the epithelial cells of that part of the canaliculi (cortical) where, from what is known of the action of the epithelium in other glands when an increase of the functional activity takes place in them, the fact might be readily supposed; but the same stimulation is also observed, and in a very distinct way, in the epithelium of that part of the canaliculi (straight canaliculi of the Malpighian pyramids, and even in the large canaliculi of the papillæ near their opening into the renal pelvis) in regard to which the opinion of a passive condition, *a priori*, would seem much more justified." Both in the first and in the second systems of canaliculi Golgi has demonstrated that, following nephrectomy of the opposite side, innumerable karyokineses are developed, which show an active cellular proliferation.

With the same object and the same criteria Beresowsky [1] studied compensatory hypertrophy of the thyroid gland following the extirpation of a part of the gland. Kraske [2] demonstrated the importance of the processes of proliferation in compensatory hypertrophy of the salivary glands; Di Mattei,[3] and later Stilling,[4] in compensatory hypertrophy of the supra-renal capsules.

From these studies it results that owing to a wonderful process of cell multiplication our organism is capable of compensating the loss, or the grave injury, of certain important organs, by the increase of the active elements of the homologous organs.

[1] Beresowsky, "Compensatorische Hypertrophie d. Schilddrüse," 'Ziegler's Beiträge,' 1892.

[2] Kraske, "Compensatorische Hypertrophie der Speicheldrüsen," 'In. Diss.,' Bonn, 1888.

[3] Di Mattei, "Sulla iperplasia compensatoria delle capsule soprarenali," 'Giornale delle Accad. di Medicina, Torino,' p. 127, 1886.

[4] Stilling, "Compensatorische Hypertrophie der Nebennieren," 'Virchow's Archiv,' vol. clxxxviii, p. 569, 1889.

Regeneration.

But the process of proliferation displays itself also in a very active way, reproducing destroyed parts, regenerating elements lost in consequence of traumatic or physico-chemical actions, or of the attacks of other living beings.

The demonstration of the laws which govern such pathological manifestations certainly encounters great difficulties, inasmuch as such laws cannot be blindly sought by studying the lower animals, and with the direct ascription of the phenomena observed in animals to the human organism.

The distribution of the regenerative faculty is very irregular in the animal kingdom. As a rule it may be said that it is the more easy the less elevated the organism of the animal. But even this concept is not by any means absolute. The regenerative faculty, which is very potent in the hydra (Trembley, Kleinenberg, R. Zoia), is still potent in the Holothuriæ, which in nine days reproduce their digestive tube that they have expelled by voluntary mutilation. The Ascididæ can regenerate even their nervous system, the Crustaceans regenerate their chelæ; the Tritons not only reproduce an amputated limb, but, according to the splendid researches of Griffini,[1] also regenerate an excised eye with its parts, including the retina. But fishes, on the other hand, have very little regenerative power, mammifers have little, and birds have still less. We cannot discuss here the whole problem of regeneration in the various species of animals, and we must limit ourselves to what concerns man only. The different tissues of which he is formed present this reparative activity in various degrees; and the mode in which this develops varies, as is very natural, according to the different constitution of the tissue. To the study of this mode and the explanation of these differences the efforts of pathologists have always been directed, and the recent researches on the proliferation of cells have thrown much light on the subject.

[1] Griffini, "Sulla rigenerazione totale della retina nei tritoni," 'Riforma medica,' Gennaio, 1889.

It is very obvious that the greatest activity takes place in those cases which, according to Bizzozero's studies, are normally the seat of continuous renewal, like the epithelia of investment, and the glands secreting morphological elements. The regenerative activity of these tissues has been recognised for a considerable time, and the new researches have proved only how such renewal takes place owing to the processes of cellular proliferation.

Very different was the case of the glands with amorphous secretion. For these the tendency to regeneration was denied by many authors; by others it was admitted as being probable only. But since in the demonstration of the karyokinetic figures we have found a certain criterion for recognising cell multiplication, then the question has been definitely solved. In fact, in 1884, Golgi,[1] studying the pathological histology of the *kidneys* in Bright's disease, showed that after the disorganisation of the renal epithelia, consequent on the regressive process by which the epithelia are extensively invaded in parenchymatous nephritis, when the disease begins to improve numerous cells are observed undergoing proliferation, and in this way complete *restitutio ad integrum* may take place.

Canalis[2] and Podwissozki[3] have studied independently the reparative processes of wounds of the *liver* and of the *submaxillary glands*, and have demonstrated that the specific elements are regenerated by the proliferation of the remaining elements.

Ribbert[4] has succeeded in extirpating five sixths of a salivary gland in a rabbit, and has observed its almost complete regeneration by the proliferation of the remaining elements.

[1] Golgi, 'Neoformazione dell' epitelio dei canalicoli uriniferi nella malattia di Bright,' Torino, v. Bona, 1884.

[2] Canalis, "Sulla reazione degli elementi delle capsule surrenali e delle ghiandole sottomascellari all' irritazione traumatica," 'Acc. di Med. di Torino,' 1885, p. 181; see also Canalis, "Intorno alla rigenerazione del tessuto epatico," 'Gazzetta delle Cliniche,' 1885, p. 129.

[3] Podwissozki, "Experim. Untersuchungen üb. d. Regeneration der Drüsengewebe," 'Beiträge v. Ziegler,' i und ii.

[4] Ribbert, "Ueber der Regeneration der Speicheldrüse," 1894, 'Archiv für Entwickelungsmechanik der Organismen,' vol. i.

Di Mattei [1] has observed regeneration following wounds of the *pancreas ;* G. Martinotti [2] having extirpated the pancreas, saw the organ regenerate by the continuous cellular proliferation of small parts of the gland left *in situ.*

Podwissozki [3] saw the *Meibomian glands* regenerate in the same way, Coen [4] the *mammary gland,* Drogoul [5] obtained analogous results in the *prostaté,* Vincenzi [6] in the *thyroid* gland.

With regard to the regeneration of the mucosa of the *stomach* we have the notable observations of Griffini and Vassale,[7] who recognised that the mucosa when removed even in large tracts was reproduced with the complete renewal of the organs that were contained there, new glands gradually developing by the proliferation of the epithelium of investment, which in its turn was derived from the glands that were nearest the solution of continuity. Vivante [8] had similar results, studying the reproduction of the pyloric mucosa. In this way it was established that whatever characters of stability the elements of these glands have in the normal state, they, however, have not lost the faculty of multiplying themselves when necessity requires it. When a gland, in any morbid process whatever, loses a large number of its elements, it can successively reproduce them and return to the normal state, as Golgi was the first to demonstrate, studying the cure of Bright's disease. This tendency to

[1] Di Mattei, "Sugli effetti della irritazione degli elementi del Pancreas," 'Acc. di Med. di Torino,' 1885, p. 476.

[2] G. Martinotti, "Ueber Hyperplasie und Regeneration der drüsigen Elemente in Beziehung auf ihre Functiontähtigkeit," 'Centralblatt für allgemeine Pathol. und path. Anat.,' i, 633—638, 1890.

[3] Podwissozki, l. c.

[4] Coen, "Zur Regeneration der Milchdrüse," Ziegler's 'Beiträge,' vol. ii.

[5] Drogoul, "Sulla rigenerazione della prostata," 'Giornale della Acc. di Med. di Torino,' 1887.

[6] Vincenzi, "Sulla rigenerazione parziale della ghiandola tiroide," 'Gazzetta degli ospedali,' N. 54, anno 1885.

[7] Griffini e Vassale, "Sulla riproduzione della mucosa gastrica," 'R. Acc. di Med. in Modena,' 1888; "Beiträge zur pathol. Anat. und allg. Pathol. von Ziegler," B. iii, 1888.

[8] Vivante, "Sulla riproduzione della mucosa pilorica," Torino, 'Real. Acc. delle Scienze,' 1894, serie 2, vol. xliv.

regenerating is so great that, as is well known, Ponfick,[1] extirpating in animals the greater part of the liver, saw it very quickly return to its normal size.

The fact (formerly placed in doubt) of the partial regeneration of the cornea, demonstrated by Mayzel,[2] Eberth,[3] Peters,[4] etc., is notable; as is that of the epithelium of the capsule of the crystalline lens, demonstrated by Falchi.[5]

All these facts have much importance also in regard to the prognosis and the treatment of certain human infirmities, as we shall see in another chapter.

The *connective tissue* possesses a very great faculty of proliferation. This has been known for a long time, especially owing to the remarkable labours of Virchow.[6] The new researches, however, on the proliferation of the connective-tissue cells in all those cases in which there is hyperplasia of the tissue demonstrate, according to Bizzozero,[7] that it is not necessary to have recourse to the transformation of the leucocytes to find the origin of the neoformed connective-tissue cells.

Bizzozero and Canalis[8] have recognised, in fact, that in wounds of the integuments not only the fixed connective-tissue cells, but also the round cells, multiply by mitosis; the karyokinetic figures are very frequent in the young connective tissue of wounds; they heal them by first or by second intention.

[1] Ponfick, "Ueber Rekreation der Leber," 'Verhand. des Xen internat. Kongresses zu Berlin, 1890,' II; 'Allg. Pathol. und path. Anat.,' Berlin, 1891.

[2] Mayzel, "Ueber eigenthümliche Vorgänge bei der Theilung der Kerne in Epithelialzellen," 'Centralb. f. d. med. Wissensch.,' S. 849, 1875.

[3] Eberth, "Ueber Kern und Zelltheilung," 'Virchow's Archiv,' Bd. xlvii, 1876.

[4] Peters, 'Regeneration des Epithels der Cornea,' Bonn, 1885.

[5] Falchi, "Riproduzione dell' epitelio del cristallino in condizioni normali e patologiche," 'Arch. p. l. Scienze mediche,' vol. iii.

[6] Virchow, 'Virchow's Arch.,' vol. v, xvi; 'Würzbürger Verhandlungen,' vols. i and ii.

[7] Bizzozero, "Accrescimento e rigenerazione nell' organismo," 'Arch. p. le Scienze med.,' vol. xviii.

[8] Bizzozero e Canalis, "Sulla scissione degli elementi nei focolai flogistici," 'Giornale dell' Accad. di Medicina di Torino,' 1885, p. 182.

Mondino,[1] studying the healing of wounds of the brain, saw the vessel endothelia, the lymphoid elements, and the neuroglia cells, multiply by karyokinesis.

The proliferating power of the connective tissue is, therefore, extraordinarily great. This tissue, as Virchow has demonstrated, is found diffused in the whole organism of which it forms the stroma of support. When a destruction of parts takes place and their regeneration is impossible, the connective tissue develops instead, and fills up the lacuna left, forming a cicatrix. The rapidity of development of the connective tissue, the mass of the cells, the extension of the process of proliferation, may be very varied; and the numerous observations of Marchand,[2] Coen,[3] Graser,[4] Roloff, Eberth,[5] etc., by means of the modern methods of histological *technique*, have proved that the regeneration of the connective tissue, the formation of the granulation tissue, exclusively occurs through the proliferation of the pre-existing connective-tissue cells. These authors, repeating the experiments of Ziegler, Tillmanns, and Senftleben on the development of the connective tissue after the introduction of foreign bodies under the skin or into other parts, have proved that the formation of the granulation cells always begins at the periphery of the foreign body, and then gradually proceeds towards the centre if the foreign body be permeable. The proliferation takes place by the karyokinesis of the surrounding connective-tissue cells, which, multiplying, gradually enter the foreign body. The signs of cellular proliferation are also found within the plugs of elements that have already entered the foreign body. Together with these elements leucocytes are found, which are due to the irritative action of the foreign body,

[1] Mondino, "Nuove osservazioni intorno all' infiammazione traumatica sperimentale del tissuto cerebrale," 'Giornale dell' Accademia di Medicina di Torino,' 1885, p. 76.

[2] Marchand, "Untersuchungen über Einheilung von Fremdekörper," 'Beiträge zur path. Anat.,' vol. iv, S. 1.

[3] Coen, "Ueber die pathologisch-anatomischen Veränderungen der Haut nach der Einwickelung von Iodtinktur," Ziegler's 'Beiträge zur pathol. Anat.,' vol. ii, p. 29.

[4] Graser, "Untersuchungen über feineren Vörgange bei Verwachsung peritonealer Blätter," 'Zeitschrift für Chirurgie,' vol. xxvii, 1888.

[5] Eberth, "Gewebsbildung," 'Fortschritte der Medicin,' 1892.

but forms intermediate between the leucocytes and the fixed cells of the tissue have never been observed.

Even Ziegler[1] now accepts this order of ideas, and thus explains his famous experiments made by the introduction of small glass chambers under the skin or into the peritoneum. That is, he maintains that the new regenerative or inflammatory production takes place by the proliferation of the fixed connective-tissue cells, without the participation of the leucocytes migrated from the blood. These leucocytes are in part destined to death; in great part they migrate anew by the channel of the lymphatic vessels, exporting by phagocytosis the useless materials which they ingest.

The young connective-tissue cells of recent formation are round or oval, with a large nucleus, and with clear, finely granular protoplasm. These young cells gradually assume a spindle shape (they become fibroblasts), and finally elaborate the fundamental substance, probably by a fibrillar transformation of their ectoplasm. In the end they become long, thin, or stellate, as in the adult connective tissue. While the young connective tissue is rich in vessels, with the development of the connective-tissue fibrils the vessels stretched by these fibrils atrophy, the tissue becomes more consistent, poor in juices, and cicatricial retraction takes place.

The neoformation of *vessels* and their regeneration are intimately connected with the regeneration of the connective tissue; furthermore, as a rule, the development of the connective tissue is always in relation with the formation of new blood-capillaries.

As is known, three principal modes of formation of vessels are admitted, one of which is observed only in the embryo, and therefore has no importance for pathology. The so-called secondary neoformation of the capillaries, admitted by Billroth and Rindfleisch, consists in the apposition of the fusiform connective-tissue cells between which a lumen is found, that afterwards is put in communication with the blood-vessels. But it is very doubtful if this mode of formation of the vessels really takes place. On the other hand, the formation of vessels by sprouts, developing from the

[1] Ziegler, "Ueber die Betheiligung der Leukocyten an der Gewebeneubildung," 'X Congress Med.,' Berlin, 1891.

parietes of the surviving vessels, has been undoubtedly demonstrated. From the cells of the wall of a capillary protoplasmatic sprouts are first formed, within which the nuclei of the pre-existing endothelial cells pass. In a more precise way, according to the minute researches of Thoma [1] and Jamagiwa,[2] these sprouts develop by the proliferation of the endothelial cells, whose nuclei divide by a process of karyokinesis, and extend from the vessel with the protoplasmatic sprouts. For the ulterior proliferation of these nuclei the prolongations lengthen, and finally meet, and thus the outline of the new channel is formed. The lumen of the vessel, according to Arnold,[3] is excavated by the liquefaction of the central part of the parietal sprouts, but Thoma has positively shown that the lumen is formed by the gradual dilatation of the intercellular spaces interposed between the neoformed endothelial elements by the force of the permanent pressure of the blood.

These new discoveries on the proliferation of the connective tissue have exercised great influence, not only on general pathology, as we shall see better when speaking of inflammation, but also on surgical pathology, inasmuch as they have demonstrated the mechanism of the healing of wounds.

The regeneration of *cartilage*, according to the latest studies, is effected in part by the proliferation of the remaining cartilage cells, in which Tizzoni [4] has observed karyokinetic figures, in part by the proliferation of the connective-tissue cells (perichondrium) which are gradually transformed into cartilage cells, elaborating a hyaline fundamental substance that contains a particular chemical substance, chondromucoid. According to Strasser,[5] it is only the connective tissue of the

[1] Thoma, 'Histogenese und Histomechanik des Gefäss-System,' Stuttgart, 1893.

[2] Jamagiwa, "Entzündliche Gefassneubildung," 'Virchow's Archiv,' vol. cxxxii, 1893.

[3] Arnold, "Ueber die Entwickelung der Blutcapillaren," 'Virchow's Archiv,' vols. liii und liv.

[4] Tizzoni, "Sulla scissione indiretta delle cellule cartilaginie nella condrite sperimentale traumatica," 'Gazzetta dei Ospedali,' 1885, p. 370; see also Sieveking, "Wachsthum und Regeneration des Korpels," Schwalbe's 'Morphol. Arbeiten,' i, 1892.

[5] Strasser, "Zur Entwicklung der Extremitätenkorpel bei Salamandern," 'Morphologische Jahrbücher,' vol. v, p. 240.

perichondrium which is capable of regenerating cartilage (together with the remaining cartilage cells). The formation of the fundamental substance is effected by the gradual transformation of the cellular ectoplasm. When the perichondrium and the cartilage are destroyed the surrounding connective tissue is not generally capable of re-forming it, and then the formation of a connective-tissue cicatrix takes place.

The regeneration of *osseous tissue* explains how the union of fractures is effected. We cannot describe here the whole process of ossification, with which the formation of callus is intimately connected. We shall summarise only the recent studies of Kráft,[1] which demonstrate that the union of fractures essentially depends on the processes of cellular proliferation. Already, two days after the lesion, numerous karyokineses are found in the periosteum and in the endothelium of the vessels in the vicinity of the injured part. On the fourth day the whole of the osteoblastic stratum of the periosteum in the neighbourhood of the fracture is transformed into a young tissue, rich in vessels and proliferating elements. This tissue is afterwards differentiated into trabeculæ which anastomose with those formed in the bone marrow; then they undergo a process of ossification. The external callus is formed altogether by the periosteum, and also the intermediate callus in great part; the internal callus, on the contrary, is formed by the proliferation of the marrow cells. This tissue, at first soft and rich in vessels, is gradually transformed into osteoid tissue. Between the cellular elements a fundamental substance is formed, which is gradually impregnated with lime salts, while the cells assume the stellate appearance of bone corpuscles.

The new discoveries acquired in relation to the proliferating capacity of the *non-striated muscular tissue* are of great pathological and clinical importance. Up till a few years ago it was not known whether a regeneration of the smooth fibres really existed; and pathologists were ignorant as to the mode in which they originated in hyperplasias and in tumours. The studies made principally in Italy by Busachi,[2]

[1] Kraft, "Zur Histogenese des Callus," 'Ziegler's Beitr.,' vol. i, p. 85.

[2] T. Busachi, "Sulla scissione indiretta delle fibre muscolari lisce in sequito ad irritazione," 'Giornale della R. Accad. di Medicina di Torino,'

Poggi,[1] Cattani,[2] and abroad by Stilling and Pfitzner,[3] and Ritschl,[4] have proved that the tendency of the smooth muscular tissue to proliferate is greater than that which was suspected, and that the production of new elements also takes place in it by mitosis of the pre-existing elements. Producing, for example, a stenosis of the intestine, already after two days the muscular coat above the stricture is thickened, the muscular fibro-cells are seen increased in size, and many of them are already undergoing division. The karyokinesis occurs along the principal axis of the fibre, the two daughter nuclei separate in their whole length, and the entire fibro-cell elongating ends by dividing into two. These processes of proliferation, observed by Busachi for the first time in mammifers, were confirmed by him in observations made on man.

With regard to *striated muscular fibres*, the results obtained up to now are very conflicting; from the researches of Perroncito,[5] Barfurth,[6] Kirby,[7] Nauwerck,[8] Volkmann,[9] etc., it results that, following the loss of substance in muscle there is a regeneration which always depends on the nuclei of the pre-existing fibres.

1886, p. 55; see also Busachi, "Ueber die Neubildung von glatten Muskelgewebe," 'Beitr. v. Ziegler,' iv, 1888, p. 101.

[1] Poggi, "Le cicatrisation immédiate des blessures de l'estomac," 'Beitr. v. Ziegler,' iii, 1888, p. 239.

[2] Cattani, "Cariocinesi nelle fibre muscolari dell' utero," 'Gazzetta degli Ospedali,' 1885.

[3] Stilling u. Pfitzner, "Regeneration glatten Muskeln," 'Virchow's Archiv,' Bd. cix, 1886.

[4] Ritschl, "Ueber die Heilung der Wunden des Magens, Darm-Kanals, und Uterus, mit besonderer Berüchsichtigung des Verhaltens der glatten Muskeln," 'Virchow's Arch.,' Bd. cix, 1887, p. 507.

[5] Perroncito, "Contribuzione alla patologia del tessuto muscolare striato," 'Giornale della R. Accademia di Medicina di Torino,' 1882; and 'Arch. de Biol.,' t. i, p. 367.

[6] Barfurth, "Zur Regeneration der Gewebe," 'Arch. f. mikr. Anat.,' Bd. xxxvii, 1891.

[7] Kirby, "Experimentelle Untersuchungen über die Regeneration des quergestreiften Muskelgewebe," 'Ziegler's Beitr.,' 1892, p. 302.

[8] Nauwerck, 'Ueber Muskelregeneration nach Verletzungen,' Jena, 1890.

[9] Volkmann, "Ueber die Regeneration des quergestreiften Muskelngewebes beim Menschen und Säugethier," 'Ziegler's Beiträge,' vol. xii, 1893, p. 233.

These nuclei give rise to two kinds of formations: the first represented by special cells—sarcoblasts,—that, according to some, are transformed into true fibres, according to others, on the other hand, they undergo a process of degeneration; the second represented by conical protoplasmic sprouts which depart from the extremity of the stumps of the divided muscular fibres, and advance as thin fibres into the granulation tissue that meanwhile is formed between the two stumps; these sprouts then assume the transverse striature, and thus lengthen the fibre by which they were generated.

But such regeneration is generally very limited, so that it cannot have importance in the functionality of the muscle; indeed, the cicatrix which finally results is almost exclusively formed of connective tissue.[1]

The *nervous tissue* presents a regenerative activity still more scarce,—in fact, almost nothing in our opinion.

Mondino,[2] making aseptic punctures in the brain, has seen certain figures in some of the nerve-cells which characterise the first phases of karyokinesis, but he has never succeeded in following these figures to the division of the cell, nor even in seeing those phases of nuclear evolution that constitute metakinesis. Coen,[3] Sanarelli,[4] and Stroebe [5] have seen the connective-tissue cells, the endothelia of the vessels, the smooth fibres of the latter, and the elements of the neuroglia segments regenerating themselves in the brain, but never the nerve-cells. Very recently Tirelli [6] has studied the reparative processes in the intervertebral ganglion, and Monti

[1] In the study of the healing of wounds of muscles we have never observed any clinical condition that truly indicated a regeneration of the destroyed tissue.

[2] Mondino, "Sulla cariocinesi delle cellule nervose consecutiva ad irritazione cerebrale," 'Rendiconto del R. Istit. Lomb.,' 1885.

[3] Coen, "Ueber die Heilung von Stichwunden des Gehirns," 'Beitr. v. Ziegler,' ii, 1889, p. 107.

[4] Sanarelli, "Sui processi riparativi nel cervello e nel cerveletto," 'Memorio dei Lincei,' 1892.

[5] Stroebe, 'Exp. Untersuchungen über die degen. und reparatorischen Vorgänge bei Heilung von Ruckenmarkswunden," 'Beiträge v. Ziegler,' xv, 1894, p. 383.

[6] Tirelli, Monti, and Fieschi, mentioned in the 'Ergebnisse der allgemeinen Pathologie und patholog. Anatomie di Zubarsch und Oestertag,' 1895, anno ii, pp. 795-6.

and Fieschi have studied the healing of aseptic wounds in the sympathetic ganglia, but none of these authors, while they saw the elements of the vessels, the connective-tissue cells, and the endothelia of the pericellular capsules proliferate, have ever seen a proliferation of the nerve-cells; on the contrary, they have observed that the healing takes place with the formation of a connective-tissue cicatrix derived from the proliferation of the elements of the capsule. Monti and Fieschi have seen some of the first phases of karyokinesis in the nerve-fibres, but they have not been able to observe a true proliferation of the fibres within the ganglion; on the other hand, according to Ranvier,[1] Tizzoni,[2] Vanlair,[3] Stroebe,[4] etc., nerve-fibres regenerate with great energy. But, observes Bizzozero, nerve-fibres are not true autonomous elements, but they certainly are a product of cellular activity, namely, simple prolongations of a cell which increase solely by the work of the cell from which they have had their first origin.

On the basis of the difference that the various tissues present as regards pathological regeneration, Bizzozero has distinguished the tissues into two groups. To the first group belong the tissues which are capable of completely regenerating themselves whether they have stable or labile elements,—that is to say, the epithelia of investment and the glands secreting morphological elements (tissues with labile elements), the epithelia of the glands with amorphous secretion, the connective tissue, the blood-vessels, the cartilaginous tissue, the osseous tissue, and the non-striated muscular tissue (tissues with stable elements). To the second group, on the contrary, belong those tissues which in adult life, once destroyed, do not regenerate themselves, and give rise to the formation of connective-tissue cicatrices. Such are the striated

[1] Ranvier, 'Leçons sur le système nerveux,' Paris, 1878.

[2] Tizzoni, "Sulla patologia del tissuto nervoso," 'Arch. p. le Scienze med.,' vol. iii, 1878; and 'Centralblatt f. d. m. W.,' 1878.

[3] Vanlair, "La persistence de l'aptitude régénérative des nerfs," 'Bull. d. l'Ac. r. d. Belgique,' 1888.

[4] Stroebe, 'Exp. Unters. über Degeneration und Regeneration peripherischen Nerven," 'Beitr. v. Ziegler,' xiii, 1893.

muscular tissue and the nervous tissue, which Bizzozero calls tissues with perennial elements.

It is not necessary to comment upon the influence of these studies on pathology and the clinic. They, in fact, tell us that in wounds of the central nervous system, as well as in those of the spinal and sympathetic ganglia, we must expect all the consequences of losses of substance, which cannot be repaired in a direct way, and only in certain cases may be compensated by a vicarious function. These studies, besides, indicate a no distant explanation of some teratological phenomena. It has already been observed in animals that when regeneration takes place in abnormal conditions, peculiar anomalies or true monstrosities develop. Thus many years ago Maggi saw a double claw develop in an *Astacus fluviatilis* [1] following an irregular amputation.

Dugès [2] obtained planariæ with two heads, dividing the animal for a certain length from before backwards; in the same way he observed individuals with two tails. Vulpian [3] has observed that the axolotl kept in aquariums have frequently one or other extremity bitten off; such extremity, as is known, regenerates, but sometimes in the regeneration it presents supernumerary digits. Girard [4] has seen acquired polydactylism in certain tritons of Spain (*Pleurodeles Waltlii*); Piana [5] has obtained the production of supernumerary digits in our tritons, making with scissors oblique wounds of the carpus or tarsus. By the same means Piana has obtained experimentally lizards with two tails.

Now the researches made by modern embryologists on lesions of the blastomeres, and on the development of the ova in abnormal conditions, undoubtedly co-ordinate with these

[1] Maggi, "Mostruosità di un gambero d' acqua dolce," 'Rend. dell' Ist. Lomb.,' vol. xiv, f. 8.

[2] Dugès, "Recherches sur l'organisation et les meurs des Planariées, 'Ann. de Sciences Nat.,' 1828.

[3] Vulpian (quoted by Delage), 'Sur l'hérédité,' Paris, 1895.

[4] Girard, "Polydactylie provoquée chez *Pleurodeles Waltlii*, Mich.," 'Compt. rend. Soc. Biol.,' ser. 10, ii, p. 789.

[5] Piana, "Ricerche sulla polidattilia acquisita determinata sperimentalmente nei tritoni, e sulle code sopranumerarie nelle lucertole," 'Ricerche del Lab. d' Anatomia di Roma,' 1894.

conditions of regeneration, and perhaps in the future we shall have a much more precise explanation of certain monstrosities by excess than that which we have at present.

INFLAMMATION.

We shall now proceed to study the influence that the doctrine of pathological proliferation has exercised on the studies of inflammation.

The doctrine of inflammation has been developing and modifying according to the development of our anatomical and physiological knowledge; and the various theories which have arisen may be considered as the reflex of the successive discoveries of anatomy and physiology.

Rokitansky,[1] having recognised the importance of the disturbances of the circulation in the various pathological processes, instituted the hydraulic theory of inflammation, according to which there is first acceleration of the current, then slowing, and finally extravasation and exudation.

Later on Virchow, having proclaimed the principle of the autonomy of the elements, attributed the inflammatory process to alterations in the activity of these elements, and made the hyperæmia depend on the increased activity of the inflamed elements, which acquire the property of attracting the circulating juices; thus occurs a greater afflux of blood to the parts. The exudate, then, according to Virchow, in its liquid part consists of the surplus quantity of the juices, in its solid part of the pathological proliferation of the tissue cells. To explain how inflammation always presents equal characters in all the tissues, Virchow made use of his studies on the diffusion of the connective tissue, and on the constant presence of numerous cells within the same. So that, finding the connective tissue or its derivatives forming the stroma of every organ of the body, it was easy to explain in what way, the inflammation localising itself especially in the connective tissue, notwithstanding the difference of the organs, the manifestations were nearly always the same.

Equally with the old Italian school of Guani, Giannini, Fanzago, and Brera—as Sangalli has justly recorded in his treatise

[1] Rokitansky, loc. cit.

—Virchow recognised that irritation is the direct incentive to every inflammation: irritation perturbs the fundamental activities of the elements; the nutritive disturbance gives rise to a turbidness and an increase of the cellular protoplasm,—that is to say, a perversion of the nutrition with a tendency to degeneration. Owing to the influence of the irritation on the formative activity of the elements there is then proliferation of these elements, especially of the fixed connective-tissue cells, and consequently the formation of numerous round cells in the exudate.

We cannot further enter into the exposition of Virchow's doctrine of inflammation, because we must limit ourselves to the consideration of the phases of the theory of cellular proliferation. Virchow's doctrine on the formation of the cells of the exudate by the heterologous hyperplasia of the pre-existing elements was not readily accepted by those authors, who had never been able directly to follow the elements in question.

So long ago as 1843 William Addison,[1] in his 'Experimental and Practical Researches on Inflammation,' expounded a theory in relation to the genesis of the exudate which agrees perfectly with that now universally accepted. Addison, in fact, wrote: "During inflammation—using the word in the general sense here indicated—there is more or less marked increase of the colourless elements and protoplasma in the parts affected. At first—in the first stage—these elements adhere but slightly along the inner margin or boundary of the nutrient vessels, and are therefore still within the influence of the circulating current; belonging, as it were, at this period as much, or rather more, to the blood than to the fixed solids. Secondly—in the second stage—they are more firmly fixed in the walls of the vessels, and therefore now without the influence of the circulating current. Thirdly—in the third stage—new elements appear at the outer border of the vessels,

[1] Addison, 'Experimental and Practical Researches on Inflammation, etc.,' London, Churchill, 1843. Dutrochet also, in his 'Recherches anatomiques et physiologiques,' published in 1824, had seen the passage of the white corpuscles through the vessels; this discovery has been recently credited to him by Howarth, 'Comptes rendus de l'Académie des Sciences,' 1884, vol. xcix.

where they add to the texture, form a new product, or are liberated as an excretion."

The merit, consequently, belongs to Addison of having formulated that doctrine which now unjustly passes under the name of Cohnheim. But there is more than this: even Cohnheim's famous experiment, to which so much importance is attributed as a proof of *diapedesis*, was performed by Augustus Waller in 1839, and was accurately described with all its details in two notes published by him in 1846.[1]

After having described the way in which the circulation in the tongue and in the mesentery of the live frog can be observed, Waller describes minutely the engorgement of the capillaries, stasis, the to-and-fro movements, the reflux currents, etc., and finally the particular mode of behaviour of the white corpuscles in the capillaries of the organs in a state of irritation. In an addition to his first note Waller wrote as follows:

"Recent observations have enabled me to decide the much-agitated question as to the formation of pus, and its origin from the extravasation of the colourless or spherical corpuscles from the capillaries" (p. 285).

And further on (pp. 286, 287) Waller describes one of his experiments as follows:

"The tongue of the live frog was distended in the ordinary way. After the observation had continued for half an hour, numerous corpuscles were seen outside the vessels, together with a very few blood-discs, in the proportion of about one to ten of the former. The extravasated particles were equally diffused over most part of the tongue. No appearance of rupture could be seen in any of the vessels. The corpuscles were generally distant, about 0 to 0·3 mm. from their parietes. After the experiment had lasted about two hours, thousands of these corpuscles were seen scattered over the membrane, with scarcely any blood-discs. The process by which they passed out of the vessel could be best observed

[1] Waller, "Microscopic Examination of some of the Principal Tissues of the Animal Frame, as observed in the Tongue of the Living Frog, etc.," 'Philos. Magazine,' vol. xxix, 1846, pp. 271 and 397; see also Waller, "Microscopic Observations on the Perforation of the Capillaries by the Corpuscles of the Blood, and on the Origin of the Pus Corpuscles," 'Phil. Mag.,' vol. xxix, 1846, p. 397.

in a capillary containing stationary blood particles. Generally, at a slight distance from it, some extravasated corpuscles could be detected, and at the nearest opposite point of the tube a small concave depression was presented. Frequently, near this depression, numerous corpuscles were collected within the tube, as if about to follow the rest which had escaped. These were frequently agitated by a movement of oscillation, which showed that there was no open point in the tube. In other spots some of these corpuscles were seen protruding half out of the vessel. Whenever the current re-occurred in a vessel presenting these appearances, the depressions and unevenness quickly disappeared, and no trace of the corpuscular extravasation could be seen, except the presence of the corpuscles themselves.

"I consider, therefore, as established, first, the passage of these corpuscles '*de toute pièce*' through the capillaries; secondly, the restorative power in the blood, which immediately closes the aperture thus formed. It would lead me too far at present to explain how I have obtained purulent matter in these animals similar in all respects to that in the human subject."

Consequently Waller had already discovered diapedesis in inflammation.

Observation and reasoning had led many pathologists, especially the Italians, blindly to follow the doctrine of Virchôw. Sangalli,[1] for example, emphasised the importance of the circulating phenomena in inflammation, and defined it "that process of morbid nutrition by which, following a stimulus that has acted on a part of the body, various disorders of the blood circulation are provoked, producing in it a sero-albuminous or sero-fibrinous transudation with small roundish cells, capable of afterwards developing into connective tissue or epithelial elements, and into their derivatives." But this definition, besides being indefinite, is incomplete in its first part, inexact in its last.

Oehl,[2] in his remarkable study on inflammation, assigned the genetic part of the process to the vessels. Starting from the

[1] Sangalli, 'La scienza e la pratica dell' Anat. patol.'

[2] Oehl, "Fisiologia dei processo inflammatorio," 'Gazz. med. Lombardo,' 1865.

principle that the nutriment of the cells is obtained from the blood, he deduced that every time the quantity of the blood which passes in a given time through a tissue is augmented, the productive activity of the elements in this tissue must be increased; but since, in phlogosis, both the local acceleration of the circulation and the increase of the mass of the effused plasma exceed the highest physiological degrees, thus the process becomes pathological, and the product departs from the type of the generating elements.

Cohnheim,[1] reviving the old discoveries of Dutrochet, Addison,[2] Waller,[2] Zimmermann, etc., has introduced the doctrine of diapedesis into science, and has explained by this means the accumulation of cells in the inflammatory exudate, denying the cellular proliferation of the tissue elements.

Yet Stricker,[3] Böttcher,[4] Recklinghausen,[5] and others, while admitting that the alterations of the circulation and the diapedesis have an essential part in inflammation, admitted also that the elements of the tissues participated in the formation of the cells of the exudate.

Against the idea that all the leucocytes and the elements of the exudate arrive in the focus from the blood-vessels by diapedesis, the objection was raised of the number, truly extraordinary, of these elements; and it was observed that the average number of the white blood-corpuscles would not be sufficient to explain the enormous number of leucocytes which are found in some profuse suppurations. Against such objections Cohnheim replied that the numeration of the white corpuscles in hæmatological observations must be inferior to the truth, because the white corpuscles tend to remain attached to the parietes of the vessels, and consequently they come out in lesser quantity when samples of blood are extracted. On the

[1] Cohnheim, "Ueber Entzündung und Eiterung," 'Virchow's Arch.,' vol. xl, p. 20.

[2] Addison, Waller, loc. cit.

[3] Stricker, 'Studien aus dem Institute für Experim. Pathologie,' Wien, 1870.

[4] Böttcher, "Esperimentelle Untersuchungen über die Entstehung der Eiterkörperchen," 'Virchow's Archiv,' 1873, vol. lviii.

Recklinghausen, 'Handbuch der allgemeinen Pathologie des Kreislaufs und der Ernährung,' Stuttgart, 1883.

other hand, he observed that in grave inflammations tumefaction of the spleen and of the lymphatic glands takes place,—that is to say, of the organs which produce the white corpuscles.

In reality, the innumerable hæmatological observations made in these latter years on the leucocytes in various diseases have demonstrated that in consequence of every inflammatory process the white corpuscles of the circulating blood notably increase.[1] The studies made on the lymphatics have demonstrated that an active proliferation of the white corpuscles occurs in these organs, as results from the researches of Flemming,[2] Paulsen,[3] and others on hyperplasia of the lymphatic organs.

Virchow's doctrine, then, has here undergone a radical modification: the pathological proliferation in the first moments of inflammation does not take place directly in the elements of the inflamed tissue, but certainly in the hæmatopoietic organs which furnish the white corpuscles destined to accumulate in the inflammatory focus. Ziegler, in fact, expresses himself as follows on the genesis of the elements of the exudate:—"The cells that accumulate in the inflamed tissues during the first hours (or the first days) of the inflammation are leucocytes migrated by diapedesis from the blood; and this applies especially to those cells which have a polynuclear or a mononuclear character; nevertheless a growth of the fixed elements is effected in the same tissue in the successive days: the elements which thus form have not, however, the character of leucocytes" (compare Ziegler, 'Lehrbuch der allg. u. speciell. Path.,' 8 Ediz., Jena, 1895, vol. i, p. 312).

Indeed, the modern researches demonstrate that a proliferation of the tissue elements positively takes place in inflammations, and on this point we have a rich literature.

[1] Compare Limbeck, 'Grundriss einer klinischen Pathol. des Blutes,' Jena, Fischer, 1896.

[2] Flemming, "Die Zellvermehrung in den Lymphdrüsen und verwandten Organen, und ihr Einfluss auf deren Bau," 'Archiv f. mikrosc. Anat.,' 1884, Bd. xxiv.

[3] Paulsen, "Zellvermehrung und ihre Belgleitungserscheinungen in hyperplastichen Lymphdrüsen und Tonsillen," 'Arch. f. mikr. Anat.,' 1884, Bd. xxiv.

We shall now record some of the most important of these observations.

Homen,[1] studying inflammation of the cornea produced by cauterisations with chloride of zinc, has observed numerous proliferations by karyokinesis of the fixed cells of the corneal tissue in the immediate vicinity of the inflammatory focus.

De Gama Pinto[2] has seen a similar process of proliferation of the conjunctival epithelial cells in many inflammatory diseases of the conjunctiva—among others in trachoma, in pseudo-membranous conjunctivitis, etc.

Giovannini[3] has made an accurate study of diverse inflammations produced experimentally on the skin, and of many spontaneous inflammatory processes of the human skin; he has met with numerous karyokineses in acuminate condylomata, in pityriasis rubra, in psoriasis, and in various other cutaneous diseases. Unna[4] has obtained analogous results in acuminate condylomata and in venereal diseases; Ostry[5] found abundant karyokineses in syphilitic papules, and in other inflammatory processes.

Petroff[6] has studied experimental arthrites in dogs and rabbits, and has demonstrated that in serous and purulent synovites karyokinetic figures appear with great constancy in various elements of the synovial capsule.

Finally, we must state that with the more delicate methods of modern histological *technique* Bizzozero first, then March-

[1] Homen, "Untersuchungen über die Regeneration der Hornhautzellen durch indirecte Kerntheilung," 'Fortschritte der Medicin,' p. 16, 1883.

[2] De Gama Pinto, "Ueber das Vorkommen von Kariokinese in der entzündeten Bindehaut des Menschen," 'Centralb. für pract. Augenheilkunde,' April, May, 1884.

[3] Giovannini, "Kariokinese der Zellen der Malpighischen schicht bei einigen pathologischen und experim. Läsionen," 'Centralbl. f. d. m. Wissenschaft,' p. 15, 1885.

[4] Unna, "Entwickelungsgeschichte und Anat. der Haut," 'Ziemssen's Handbuch,' Bd. xiv, 1894.

[5] Ostry, "Ueber den Befund von Kariokinese in entzündlichen Neubildungen der Haut der Menschen," 'Centralbl. f. d. m. Wissenschaft,' 1883, p. 305.

[6] Petroff, "Zur Lehre von der acuten Gelenkentzündung," 'Internationale Klinik,' 1886, p. 1.

and[1] and his pupils on the one hand, Ribbert[2] and his pupils on the other, have demonstrated that during inflammatory processes an active proliferation of the fixed connective-tissue cells generally occurs. From the complex of these observations it results that the large polynuclear elements which are found in the inflamed tissue during the first period of the inflammatory process, and which form the principal part of the cells of the exudate, are leucocytes migrated from the vessels by diapedesis in Cohnheim's sense; while the cells of neoformation or the granulation cells, that are constantly found in every inflamed tissue, are derived from the proliferation of the fixed connective-tissue cells.

Our personal observations also confirm the statement that the large polynuclear leucocytes, or with the sausage-shaped nucleus absolutely prevailing, are found in the first stages of acute inflammation. These cells are easily distinguished from the connective-tissue elements, whose nuclei are roundish or oval, and frequently of a vesicular aspect. The polynuclear cells infiltrated by diapedesis are usually scattered in the tissue, or accumulated in a notable mass round the irritant material, especially if this be formed of micro-organisms. The young cells, on the contrary, which are developed by the proliferation of the fixed connective-tissue elements, are frequently arranged in lines and piled on one another, so that they have an epithelioid appearance; not rarely among these lines of cells other cells are observed which, instead of being roundish, are spindle-shaped, or intermediate between the round and the fusiform; sometimes a stellate form is seen. In these cells typical karyokinetic figures are frequently observed, which are never met with in the migrated polynuclear cells. Only the largest cells derived from the proliferation of the connective tissue can be confounded with the mononuclear leucocytes. As is known, the large mononuclear cells of the circulating blood do not participate in the diapedesis; besides, they are very scarce forms: on the other hand, the small mononuclear leu-

[1] Marchand, "Untersuchung über die Einheilungen von Fremdkörpem," 'Beiträge v. Ziegler,' Bd. iv, 1889, p. 1.

[2] Ribbert, "Ueber die Betheiligung der Leukocyten an der Neubildung des Bindegewebes," 'Centralbl. f. allg. Pathol.,' i, 1891.

cocytes, the so-called lymphocytes, do migrate from the vessels into the tissues. According to Ribbert,[1] such forms are also derived from the fixed cells of the tissue, as occurs in the lymphatic glands; they are observed, as a rule, in the advanced stages of inflammation, and this is in favour of the idea that they are derived from the fixed connective-tissue cells, especially in chronic hyperplastic inflammations. Precisely in these forms of inflammation small cells of a lymphoid type undergoing karyokinesis are found in the focus.

Nevertheless modern authors, with Ziegler[2] and Birch-Hirschfeld, admit a distinction between the migratory cells that form the exudate proper, and the proliferating cells of the tissue.

The latter are destined to the inflammatory neoformation; the others are destroyed, or re-enter the vessels through the lymphatics.

To the inflammatory neoformation of the connective tissue correspond the proliferations already mentioned by us in other tissues, *i. e.* the epithelia of the mucosæ, the cells of the Malpighian mucous stratum, the glandular cells; and though one must distinguish the migratory elements as an essential characteristic of the inflammatory process, he cannot distinguish by this the inflammatory proliferation of the tissue, inasmuch as this proliferation also represents a part of the reaction of the tissue against the irritation which was the cause of the phlogosis.

Admitting this idea, it is readily understood that a certain connection must exist between inflammation and the production of morbid-tissue tumours.

TUMOURS.

Virchow has extended the doctrine of proliferation also into the field of the morbid tissues; he here developed his theory of substitutive neoformation in comparison with the exudative, describing the destructive nature of neoplasms in respect to the pre-existing normal tissues. In agreement with the ideas held in the period when the doctrine of

[1] Ribbert, loc. cit.

[2] Ziegler, "Entzündung," Eulenburg's 'Realencyclopädie,' 1894.

omnis cellula e cellulâ arose, Virchow admitted *homologous* neoplasms and *heterologous* neoplasms. He, however, immediately separated himself from Lobstein's original idea, inasmuch as he interpreted heterology as the expression of the particular structure of a tumour essentially different from that of the tissue in which the tumour developed. He, in fact, wrote, "Not only should the malignant neoplasms be called heterologous, but also every tissue that differs from the known type of the part, while that neoplasm is called homologous which reproduces the primary type." Virchow's idea of heteroplasia in relation to pathological proliferation was derived from his ideas on the matrix of malignant tumours, and especially of cancer. He had observed the infiltration of small cells (migrating leucocytes) in the neighbourhood of a tumour and in its stroma, and he maintained that such small cells were the first phases of development of the cancer elements. He therefore maintained that inasmuch as the connective tissue, according to him, was the matrix of the cells of the exudate, so also it must be the matrix of the cancer cells; he called *heteroplasia* precisely the formation of the parenchymatous cells of the cancer from the connective-tissue cells,—that is to say, the transformation of one species of cell into cells of another species.

It is useless to point out that later studies on cellular specificity demonstrated Virchow's error, which was evidently due to the insufficiency of his means of observation and of the knowledge of that time. Nowadays the terms homoplasia and heteroplasia are almost universally abandoned, or they are used with a very different signification. Now by the name of heteroplasia is understood the development of a morbid tissue in a seat where a similar tissue is not normally found, and the tendency of this tissue to spread to the damage of the normal tissues. The clinical meaning of the term, however, remains almost the same, because by the name of homoplastic benign tumours are indicated; by the name heteroplastic, on the contrary, malignant tumours are indicated.

Among the heteroplastic tumours, Virchow[1] distinguished

[1] Virchow, 'Cellularpathologie,' Berlin, 1858, chap. iii; see also Virchow, 'Krankhaften Geschwülste,' Bd. i, chap. ii; idem, "Zur Ent-

two principal forms, that is the sarcomata and carcinomata; and he defined carcinomata as alveolar tumours with a stroma and a parenchyma. The stroma and the parenchyma, however, are both derived, according to Virchow, from the connective tissue in consequence of the heteroplasia, though the parenchyma presents a structure very different from that of the stroma, so that the tumour appears constituted of two distinct tissues (*organoid* tumour). Sarcomata, on the other hand, according to Virchow, are *histoid* tumours, that is constituted of a single category of elements with an intercellular substance.

Remak[1] already had had the idea that the parenchymatous cells of cancer were elements of epithelial origin, and therefore he never wished to adopt Virchow's idea, that the connective tissue was the matrix of cancer cells. Later researches made by many authors, and among the first by Thiersch[2] and Waldeyer, demonstrated the error contained in Virchow's theory. Thiersch studied the histology and the histogenesis of cancroid of the skin, and showed that its elements are derived from the deep cells of the epidermis, while the connective tissue participates only in the formation of the stroma.

Some years later Waldeyer[3] took up the argument, and demonstrated that many carcinomata of internal origin are also developed from epithelial elements; he precisely described the cancers of glandular origin.

Later Klebs,[4] Perls,[5] and Birch-Hirschfeld[6] confirmed and generalised the studies of the preceding authors, which were found in perfect agreement with the isolated observations of the older authors, among whom I am pleased to mention

wickelungsgeschichte des Krebses," 'Virchow's Archiv,' Bde. i, ix, S. 4; 'Verhandl. der Phys.-med. Gesellschaft in Würzburg,' i, 1850.

1 Remak, 'Deutsche Klinik,' Bd. vi, 1854.

2 Thiersch, 'Der Epithelialkrebs,' Leipzig, 1865.

3 Waldeyer, "Die Entwickelung der Carcinome," 'Virchow's Arch.,' vol. xli, p. 460, and vol. lv, p. 67.

4 Klebs, 'Handbuch der pathologischen Anatomie,' 1876.

5 Perls, 'Lehrbuch der allgemeinen Pathologie,' Bd. i, Stuttgart, 1877.

6 Birch-Hirschfeld, 'Lehrbuch der pathologischen Anatomie,' 1876.

Sangalli,[1] who had described the transformation of the hepatic cells into the cells of infecting epithelioma. Among the more recent authors who have studied the origin of cancer, we mention Hauser,[2] who followed the development of the cylindrical epithelial cells of cancer of the stomach and of the intestine.

Immediately after the studies of Thiersch and Waldeyer, an analytical study of the various neoplastic forms was made, which led to a more exact delimitation of the various forms of tumours.

The Germans erroneously attribute to Wagner the first description of endothelioma; this merit belongs to Golgi,[3] who in 1869, studying the structure and development of psammomata, demonstrated the endothelial origin of these tumours, and proposed for the first time the name of *endothelioma*, afterwards accepted by Uhle, Wagner, etc. Wagner,[4] studying other forms of endotheliomata of the serosæ, recognised that they really are tumours of connective-tissue origin with an alveolar structure, which correspond in their histogenesis to the first idea that Virchow had of cancer. Such tumours, however, could no longer be confounded with true epithelial carcinomata, and the opinion gradually arose that cancers are of epithelial origin, and that sarcomata and endotheliomata are of connective-tissue origin. Sangalli[5] also, who at first adopted the term cancerous tumour to indicate every malignant neoplasm, later, on the basis of ulterior studies, substituted for that term the expressions epitheliomata, sarcomata, enchondromata, infecting myomata.

The more precise classification of the various forms of tumours found support in the studies on the proliferation of cells.

[1] Sangalli, "Osservazioni e raffronti sulla patologia dei tumori," 'Rendiconti dell' Istituto Lomb.,' fasc. 70, 1886.

[2] Hauser, 'Cylinderepithelialcarcinom des Magens und des Darmes,' Jena, 1890.

[3] Golgi, "Sulla struttura e sullo svilluppo degli psammomi," 'Annali universali di Medicina,' 1869.

[4] Wagner, 'Arch. für Heilkunde,' vol. ii, 1870.

[5] Sangalli, "Osservazioni e raffronti sulla patologia dei tumori," 'Rendiconti dell' Istituto Lomb.,' 1886.

The new methods of *technique*, which have enabled pathologists actually to observe cell multiplication and to distinguish the proliferation of the various elements with the most absolute certainty, have exercised great influence on the development of the doctrine of tumours.

Arnold,[1] with the aid of the new methods, demonstrated the proliferation of cancer cells, and also met with the karyokinetic processes in sarcomata. Martin[2] made similar observations; Cornil[3] very accurately described the diverse forms of indirect division of the nuclei and of the epithelial cells in various forms of tumours, and was the first to recognise that karyokineses are regular in papillomata, but frequently atypical in carcinomata. Martinotti and Olivia[4] also gave an interesting contribution to the study of the indirect division of the nucleus in tumours; observations which fully accord with those of Filbri,[5] Rosow,[6] Podwissozki,[7] Pfitzner,[8] Aoyama,[9] etc. Babes[10] has seen karyokinesis in sarcomata; Blonski[11] ascertained the existence of processes of indirect nuclear proliferation in leiomyomata and fibromata; Arnold observed them in myxomata and enchondromata; Kosinski[12] in adenomata, carcinomata, and sarcomata.

[1] Arnold, "Beobachtungen über Kerntheilung in den Zellen der Geschwülste," 'Virch. Arch.,' Bd. cvi, 1886, p. 279.

[2] Martin, "Zur Kenntniss der indirecten Kerntheilung," 'Virchow's Arch.,' Bd. lxxxvi, 1881, p. 97.

[3] Cornil, "Sur le procédé de division indirecte des noyaux et des cellules épithéliales dans les tumeurs," 'Arch. de phys.,' 1886.

[4] Martinotti ed Olivia, "Sulla divisione indiretta nei neoplasmi," 'Accad. Med. Torino,' 1887.

[5] Filbri, 'Ueber indirecti Zelltheilung in pathologischen Neubildungen,' Bonn, 1887.

[6] Rosow, 'Rodent Epithelioma from the Clinical and Anatomico-pathological Points of View,' Petersburg, 1888 (in Russian).

[7] Podwissozki, 'Wratsch,' 1889, p. 52 (in Russian).

[8] Pfitzner, "Zur pathologischen Anatomie des Zellkerns," 'Virchow's Arch.,' vol. ciii, 1886.

[9] Aoyama, "Indirecte Kerntheilung in verschiedenen Neubildungen," 'Virch. Arch.,' cvi, 1886, p. 568.

[10] Babes, quoted by Lukjanow, 'Allg. Pathol.,' 1891.

[11] Blonski, 'On the Development of Myomata of the Uterus,' Petersburg, 1889 (in Russian).

[12] Kosinski, 'Coloration of the Nuclei at Rest and in Mitosis of the Adenomata, Carcinomata, and Sarcomata,' Wratsch, 1888 (in Russian).

We could quote many more studies made up till now on cell multiplication in tumours, but we limit ourselves to stating that Lustig,[1] Galeotti,[2] Hansemann,[3] Stroebe,[4] have found that the karyokineses in malignant tumours, instead of presenting themselves under a regular form, appear atypical. This forms, according to Hansemann, a characteristic sign of this category of neoplasms.

All these studies on pathological proliferation, besides giving a precise idea of the development of morbid-tissue tumours, have also demonstrated that the doctrine formerly admitted in a theoretic way of the transformability of the migratory cells and of the fixed connective-tissue cells into elements of varied nature is unsustainable. From the methodic comparison of the processes of pathological proliferation with the multiplication of the normal elements has resulted the law formulated for the first time by Bard, according to which all the cells of neoformation are derived from the proliferation of elements of the same nature—*omnis cellula e cellulâ ejusdem generis.*[5] The derivatives of the various layers separated in a very early embryonic period can form only tissues which have the same origin, that is which belong to the same layer. In no case, by no pathological condition, can an epithelial cell transform itself into the elements of cartilage, bone, or connective tissue; and, reciprocally, the connective-tissue cells are not capable of producing a glandular cell, or an epithelial cell of investment. The old opinion of Virchow, according to which the connective tissue was the matrix of the most diverse tissues, and gave rise to the genesis of the parenchymatous elements of epithelioma, has found no support in the numerous objective researches subsequently made by innumerable authors.

[1] Lustig und Galeotti, "Cytologische Studien ueber pathologische menschliche Gewebe," 'Ziegler's Beiträge.'

[2] Galeotti, 'Beitrag zum Studium des Chromatins in den Epithelzellen der Carcinome," 'Ziegler's Beiträge zur pathol. Anat. u. allg. Path.,' vol. xiv, p. 249.

[3] Hansemann, "Ueber pathologischen Mitosen," 'Virchow's Arch.,' vol. cxxiii, 1891, p. 356.

[4] Stroebe, "Zur Kenntniss verschiedener cellulärer Vorgänge und Erscheinungen in Geschwülsten," 'Ziegler's Beiträge,' 1891.

[5] Bard, "Le spécificité cellulaire," 'Arch. de Physiol.,' vol. vii, 1886.

Nowadays it has been demonstrated that each tissue, whether it be normal or pathological, can only generate a similar tissue, or a tissue which has a strict relationship with it.

Within these limits only is a transformation of tissue possible, and thus only can a *metaplasia* take place. According to Hansemann[1] and many others who have studied cytodiëresis and embryonic development,[2] during the development of the ovum with the progressive differentiation of the tissues the nuclei, which are the *chlerophores* (transmitters of hereditary characters), undergo a simplification; that is to say, they preserve only that idioplasm which confers a specific character on the cell, so that it is capable of forming elements of a determinate character only. According to Hansemann this cellular specificity expresses itself not only in the particular structure of the cell at rest, but also in the mode of the proliferation, inasmuch as in the various species of cells the process of karyokinesis presents specific differences which enable us to recognise the different species of elements by the form of their mitosis. For some time embryologists have recognised that, in the segmentation of the ovum, the blastomeres present a constant number of chromosomes; this number varies for the different species, so that from the number of chromosomes one can in many cases recognise to which species the ovum belongs.[3] Once the various tissues are differentiated, the number, size, and form of the chromosomes vary according to the tissues, and in such differences lay their constant specific characters.

Thus it has been demonstrated that the morbid-tissue tumours originate from a proliferation of the cells of the normal tissues; and when there are tumours of the organoid type according to Virchow (malignant epithelioma), the parenchyma of the tumour is derived from the parenchyma of the normal organ, the connective-tissue stroma is derived from a growth of the stroma of the organ, and is accompanied by neoformed

[1] Hansemann, 'Studien über die specificität, den Altruismus, und die Anaplasie der Zellen,' Berlin, Hirschwald, 1893.

[2] Häckel, 'Ursprung und Entwicklung der thierischen Gewebe,' Jena, 1884.

[3] Boveri, 'Zellenstudien,' Jena, 1890, Heft 3, p. 60.

vessels. Both the development of the parenchyma and of the stroma is effected by the karyokinesis of the pre-existing elements; the vessels are formed according to the processes of proliferation already described in other articles. With the development of the vessels during the evolution of the tumour, a more or less intense diapedesis of leucocytes may take place. This diapedesis, however, is not by any means constant, and it only expresses the effect of the irritation exercised by the tumour: the leucocytes do not in the least participate in the formation of the neoplasm; they may migrate anew, nevertheless they are in great part destroyed. But the characteristic fact which is observed in the proliferation of the elements of the tumour is that, while in the stroma the karyokineses are typical, regular, identical with those of the stroma of the organ of origin, in the parenchyma, on the contrary, the mitoses differ more from those of the matrix the more marked the atypical development in the tumour.[1]

From the verification of this fact two fundamental concepts are derived: the one is that the cause of the deviation of type must be sought in the alteration of the process of proliferation; the other is that in epitheliomata the principal morbid tissue is the epithelial parenchyma, while the stroma represents only a secondary tissue developed in consequence of the irritative action exercised by the morbid tissue.

Atypical karyokineses, therefore, form the characteristic sign of those morbid-tissue tumours which deviate from the type of tissue from which they have started. In fact, in hyperplastic and inflammatory regenerative processes the karyokineses are quite regular, and identical with those that are observed during the development and the renewal of the normal tissues. In malignant tumours, on the other hand, hyperchromatic and hypochromatic mitoses are constantly met with. The mitosès which are observed in the normal tissues of a given animal species, and which always have a definite number of chromosomes, are called *normochromatic.* The hyperchromatic mitoses have a greater number of

[1] Hansemann, 'Studien über die Specificität,' etc., Berlin, 1893.

chromosomes, which are also larger and more irregular in form and disposition. The mitoses are called *hypochromatic*, whose chromosomes are fewer in number, smaller, irregular, and less stainable than the normal.

The hyperchromatic mitoses are frequent in epitheliomata and in infecting sarcomata: they often have an extraordinarily great number of chromosomes, that sometimes exceeds a hundred. Such forms may undergo simple divisions, or even multiple karyokineses. The result of such multiple karyokineses may be the formation of three or four cells with normal, or even hypochromatic chromosomes; in other cases the karyokinesis is not entirely completed, but is limited to a proliferation of the nuclei, which gives rise to the formation of polynuclear epithelial giant-cells. The hypochromatic cells may take origin either from a multiple karyokinesis, or from an asymmetrical karyokinesis, or from the atrophy of some chromosomes. The asymmetrical karyokineses have been specially described in sarcomata.[1]

We have said that such a deviation from the normal type of mitoses, expressing a variation of the cellular type of the elements of origin, is observed only in the malignant neoplasms.

Nevertheless even here *natura non facit saltus*: so that mitoses altogether different from those of the tissue of origin are not by any means readily found in a carcinoma. Furthermore, the less the neoplastic tissue deviates from the tissue of origin, the less different the karyokineses. In cancroids, which at the beginning differ very little in their structure from the normal or hypertrophic epidermis, mitoses similar to those of the normal epidermis are always met with. Thus in adenomata of the stomach, the intestine, the trachea, the mamma, atypical karyokineses are very rarely found. They also are never met with in benign papillomata of the larynx, nose, anus, etc. The differences, however, are greater in proportion to the deviation of the morbid tissues from the type of the matrix. Moreover in medullary

1 Vitalis Müller, "Celluläre Vorgänge im Geschwülste," 'Virchow's Arch.,' cxxx, 1892, p. 512; Ströbe, "Ueber Kerntheilung und Riesenzellenbildung in Geschwülsten und Knockenmark," 'Beiträge v. Ziegler,' vol. vii, 1890.

cancers the mitoses are not only atypical in respect to those of the matrix, but are also very different from one another; in fact, according to Hansemann, the characteristic of such tumours is precisely the great variability of their mitoses.

Such is the doctrine maintained by Hansemann,[1] Hauser, and other authors. We, however, must immediately state that even this particular study on the mode of development of the pathological proliferation in neoplasms does not explain their origin.

What is the origin of tumours?

There are to-day two doctrines which oppose each other, and which are founded upon particular ideas. The one is known as the *theory of hereditary predisposition*, and is generally called *Cohnheim's hypothesis*. The other, formerly held by the old observers, and nowadays revived with a new course and on the basis of new researches, is the *parasitic theory*.

The hypothesis of congenital predisposition should be truly called the theory of Durante,[2] because it was Durante who one year before Cohnheim revealed in the most exact scientific form that doctrinal idea which, if it has given rise to infinite controversies, has nevertheless been fruitful in important studies for the doctrine of tumours. Durante, having observed that tumours frequently arose in maternal nævi, sought the anatomo-physiological cause of this fact, which was already practically known to many surgeons, and he arrived at the following conclusions:—"*The elements which have preserved their embryonic anatomical characters in the adult organism*, or which have re-acquired them by a change in their chemico-physiological activities, represent, in my opinion, *the generating elements of every neoplasm proper, and especially of the malignant neoplasms*. Such elements remain among the normally developed tissues for years and years without giving an inkling of their existence, when an irritation, a simple stimulus, is sufficient to awaken in them that

[1] Hansemann, l. c.; Hauser, l. c.

[2] Durante, "Nesso fisio-patologico tra la struttura dei nei materni, e la genesi di alcuni tumori maligni," 'Archivio di memoire ed osservazioni di Chirurgia pratica di Palasciano,' vol. xi, No. 6, May, 1874.

movement and those cellular properties which heat excites in the elements of the germinal spot of the bird's egg, which has remained inactive from the moment it was laid. Indeed, if we think for a moment how complicated the disposition of the tissues is which contribute to the organic construction of an epithelial cancer, we must admit at least that the genesis of this cancer in a limited spot of the organism is due to the *reproduction of entire embryonic acts,* represented by cells that possess the necessary anatomo-physiological properties."

In this short summary Durante clearly indicated the theory which has passed into science unjustly associated with Cohnheim's name. Within the last few years Ribbert has sketched a modification of this doctrine, which, owing to his researches, has been well received.

Cohnheim,[1] in his classic treatise on 'General Pathology,' after having confuted the opinions up to then held on the etiology of tumours, confirmed the theory of embryonic predisposition, endeavouring to strengthen this hypothesis not only with arguments by exclusion, but also by recording cases of hereditary transmissibility of tumours, their congenital appearance, etc. Cohnheim thought that "in an early stage of embryonic development a number of cells greater than that necessary for the structure of the corresponding part was produced in such a way that some of such cells were not utilised. These may be very small in amount, but owing to the embryonic nature of the cells of the part they retain great proliferative capacity." Reasoning from this hypothesis, Cohnheim believed that "one was involuntarily induced to recognise the time of this excessive production of cells already in a very early stage, perhaps in the period of development between the complete differentiation of the germinal layers and the formation of the rudiments of the individual organs; at least, in this mode it seems to me to be very easy to understand why later on from this defect there results not an enormous growth of a part of the body, but a histoid tumour only, that is the excessive

[1] Cohnheim, 'Vorlesungen über allgem. Pathologie,' Berlin, Stirschwald, 1877, pp. 634, etc.

growth of one of the tissues of a given part of the body. It is possible, besides, that the excessive cellular material is either more or less regularly spread over one of the first histogenetic germinal formations, or remains, so to speak, circumscribed at one spot. The latter fact would constitute the *local disposition* of an organ, or even of a determinate region of this organ, to the final development of a tumour; and the former, on the contrary, the disposition of a system,—as, for example, the skeleton or the skin," etc. After making some reservations on the value of these facts, Cohnheim concludes that "the cause proper which later on gives rise to the development of a tumour must be sought *in an aberration, an irregularity of embryonic disposition.*"

To strengthen his hypothesis Cohnheim called attention to teratomata and dermoid tumours, which clearly originate from aberrant elements. He also mentioned that many tumours, such as dermoids, developed in determinate spots; that others, as myomata of the prostate, rhabdomyomata of the urogenital tract, are found where the embryogenetic processes are particularly complicated, where fissures or invaginations of tissues are formed. Carcinomata find their favourite sites at the orifices of the body, where a dispersion of the elements can easily take place. Finally, Cohnheim stated that his hypothesis would explain the multiplicity of certain tumours and the frequency of myomata of the uterus, inasmuch as the uterus contains an abundant material which in determinate physiological conditions takes on an active development, and therefore can easily multiply and form tumours. To explain the cause of the development of tumours at different ages, Cohnheim thought that the aberrant germs, quiescent for a variable time, find the stimulus for their development in an increased amount of nutriment or in a diminution of the resistance of the surrounding tissues.

The Durante-Cohnheim theory has given rise to interminable discussions; it has found many supporters and an equal number of opponents.

Among the numerous objections, the chief one is that nobody has ever demonstrated the actual existence of the hypothetical aberrant germs in the organism. The smallness of

these germs, the technical difficulties which were opposed to their discovery, were the only excuses brought forward to justify the absence of the proof of their existence. But now-a-days such objections have definitely disappeared, since W. Roux,[1] in 1888, succeeded in demonstrating in the frog's embryo the existence of aberrant undifferentiated spheres of segmentation in the middle layer, and more rarely in the internal layer. He found cells having the characters of those of the *morula* or of the young *blastula* clearly recognisable by their vitelline granules, the central pigment, and the nucleus very poor in chromatin, sometimes isolated beside the medullary tube or in the midst of the tissue under the epidermoidal surface. On one occasion he recognised thirteen such elements scattered among other cells normally developed.

Roux also demonstrated that the described aberrant germs are, in fact, constantly present in the eggs of the frog, which are tardily fecundated. He finally succeeded in experimentally reproducing the phenomenon by subjecting the eggs to a certain degree of pressure. He maintains that similar anomalies of development may occur also in the human ovum when it remains for a long time in the uterus before being fecundated.

Roux has not forgotten to state that these finds of his have much importance in explaining the genesis of tumours from aberrant embryonic germs, according to the Durante-Cohnheim hypothesis.

This hypothesis is also supported by the researches of Barfurth,[2] who, studying the regeneration of the germinal layers, observed that when the eggs are punctured in the gastrular stage some of the cellular groups are pushed into the cavity of the sphere, where they are not destroyed, but may finally develop into dermoid productions.

Nor are experiments wanting which demonstrate the possibility of the development of tumours from grafts of embryonic

[1] W. Roux, "Beiträge zur Entwicklungsmechanick des Embryo," 'Virchow's Archiv,' vol. cxiv, fasc. 1, 2, 1888. See also W. Roux, "Demonstration verspvengeter persistieven der Furchungszellen in der Geweben von Embryonen," 'Centr. f. allgem. Pathol.,' 1894, vol. v, p. 858.

[2] Barfurth, 'Anatomische,' Heft 9.

tissues. Zahn[1] first, and later Leopold,[2] after having observed the incapacity of adult tissues transplanted from one organism to another to proliferate, have seen, on the contrary, that fœtal tissues, and among these especially cartilages, show great vitality. Nevertheless such fœtal grafts have never persisted indefinitely, and have always been finally reabsorbed. Ribbert[3] believes that this has taken place, because the transplanted tissues had found the conditions of existence too diverse and unsuitable for their development. We must not forget the experiments of Féré,[4] who inserted under the skin of chicks the blastoderms of the egg, embryos and fragments of embryos of the fowl, and obtained the production of various teratomata, some of which perfectly reproduced the structure of tumours and complex tissues similar to ovarian and testicular teratomata.

However great may be the importance of these new researches in upholding the Durante-Cohnheim doctrine, nevertheless many authors believe that this doctrine is applicable only to certain kinds of tumours,—in other words, they maintain that the etiology of tumours is not one, but many. In this sense Birch-Hirschfeld,[5] Ziegler,[6] and Thoma[7] express themselves in their treatises.

But Ribbert[8] has taken up the question from a new point of view, again giving a single explanation for all autonomous reproductions, and according to Barfurth's[9] opinion he has

[1] Zahn, "Sur le sort des tissus implantés dans l'organisme," 'Compt. rend. du Congrès internat. de Génève,' 1878.

[2] Leopold, "Exper. Untersuchungen ü. die Aetiologie der Geschwülste," 'Virchow's Archiv,' vol. lxxxv, 1881.

[3] Ribbert, "Ueber die Entstehung der Geschwülste," 'Deutsche med. Wochenschrift,' 1895.

[4] Féré, "Sur la production expérimentale des tératomes," 'Archives d'anatomie microscopique,' 1897—8.

[5] Birch-Hirschfeld, 'Grundriss der allgemeinen Pathologie,' Leipzig, 1892.

[6] Ziegler, 'Lehrbuch d. pathol. Anatomie,' 8th ed., 1895.

[7] Thoma, ibid., vol. i, 1894.

[8] Ribbert, "Ueber die Entstehung der Geschwülste," 'Deutsche med. Wochenschrift," 1895. Idem, "Ueber Rückbildung an Zellen und Geweben, und über die Entstehung der Geschwülste," 'Biblioteca medica,' Stuttgart, 1897.

[9] Barfurth, "Regeneration und Involution," in Merkel und Bonnet, 'Ergebnisse der Anatomie,' vol. vii, Wiesbaden, Bergmann, 1898.

succeeded in giving a solid objective foundation and a much wider interpretation to the old Durante-Cohnheim theory.

According to Ribbert, "the genesis of all neoplasms must be sought in the separation of individual cells or groups of cells from their normal positions, and in the consequent cessation of the obstacles which are opposed to their indeterminate development. The displaced elements, withdrawn from the general influence of the organism, multiply autonomously, and behave themselves as parasites, inasmuch as they develop at the expense of the organism. In tumours which consist of various kinds of tissues which cannot be derived from one another by metaplasia, it is necessary to admit that the belated germ was of a complex nature."

Ribbert, therefore, believes that no fundamental difference exists between the tumours developed from aberrant embryonic elements and those which arise from the aberration of elements which occurred during extra-uterine life. Both require special conditions for their development. Tumours are not generated in parathyroids, accessory spleens, or succenturiated livers, because the structure of the separated part does not differ from that of the principal organ. The same may be said of accessory supra-renal capsules, where tumours sometimes develop under determinate conditions.

When a physiological organ has reached its greatest development, corresponding to the normal conditions transmitted by heredity, all the parts of the organ are in a state of opposed tension, by which term is understood the sum of the reciprocal influences which the elements forming the organ exercise on one another. The greater the alteration of structure in the aberrant parts, the less the equilibrium of tension of the tissue. Consequently the power of proliferation will persist longer than the normal. To have such aberrant germs a considerable displacement of the elements is not necessary; the groups of elements separated from others may even remain in the organ. Neither is it necessary in every case that the aberrant germs should develop and produce a neoplasm; on the contrary, they may frequently undergo a process of atrophy when they do not possess sufficient energy to develop, or when they do not obtain sufficient nutriment in their new seat, or when they

meet with very great resistance from the surrounding tissues. But if this do not occur the aberrant germs will, sooner or later, slowly or rapidly, renew their multiplication and form a tumour. According to Ribbert, therefore, the characteristic note of the aberrant germs which produce neoplasms consists not so much in their embryonic character as in their detachment from organic correlation. So long as such union persists the elements multiply themselves according to physiological laws. In support of his doctrine Ribbert cites a series of observations and experiments, partly original and partly collected with great diligence from the rich field of contemporary literature. The recent studies of Ribbert have been received with much favour by pathologists, and have given an impetus to new and interesting researches.

One of the points still most discussed is the genesis of cancers. According to Ribbert, the fundamental fact is the separation of the individual epithelial cells produced by a growth of the connective tissue, and the consequent indeterminate proliferation of the epithelial germ detached from the system in equilibrium. The primary factor is, therefore, the connectival multiplication; the secondary, on the contrary, is the proliferation of the epithelium.

These views have been warmly combated by Hauser,[1] Hansemann,[2] and Hanau, who maintain that the primary factor in the genesis of cancer is the characteristic alteration of the epithelium, its tendency to indeterminate development; the inflammatory growth of the connective tissue may be altogether absent, and when it exists it is a secondary phenomenon. The alteration of the epithelial type is connected with morphological changes of the cellular body, and above all of the nucleus and its processes of fission.

This controversy is not yet ended; nevertheless the old theory of Durante-Cohnheim in its *ensemble* again occupies the field.

According to Sangalli,[3] the origin of tumours must be

[1] Hauser, "Neuere Arbeiten über Carcinom," 'Centralblatt f. allg. Pathol.,' vol. ix, 1898.

[2] Hansemann, 'Die mikroskopische Diagnose der Geschwülste,' Berlin, Hirschwald, 1897.

[3] Sangalli, 'La scienza e la pratica dell' Anat. patol.,' lib. v, fasc. 1.

sought in a process of perverted local nutrition; and such idea perfectly agrees not only with the doctrine of pathological proliferation in general, but also and in a special manner with Hansemann's theory, inasmuch as the perversion of nutrition may explain how the deviation in type of the mitoses occurs, and therefore the *anaplasia* of the cells.

But what is the agent that gives rise to this perversion of the local nutrition? Many contemporary observers have endeavoured to solve this problem, searching for special parasitic micro-organisms in the neoplastic tissues. Numerous works have already been published on the argument; thus Darier [1] has searched for psorosperms in Paget's disease, Syöbring,[2] Kerotneff,[3] Pfeiffer,[4] and others have described protozoa in cancer. Ruffer [5] and Foà [6] have given very minute descriptions of particular elements included in cancer cells, and they maintain that the parasitic nature of cancer has been positively demonstrated, ascribing the relative parasite to the class of the sporozoa.

On the other hand, investigators such as Sanfelice,[7] Roncali,[8] etc., have described saccharomycetes not only in carcinomata, but also in sarcomata, both in the primary and in the metastatic forms. But others have searched for saccharomycetes in tumours, and have not succeeded in meeting with them there. We also have searched for them in vain

[1] Darier, 'Sur une nouvelle forme de psorospermose en maladie de Paget,' Soc. Biol., 1889.

[2] Syöbring, "Ein parasitärer protozoenartiger Organismus in Carcinomen," 'Fortschritte d. Medicin,' No. 14, 1890.

[3] Kerotneff, "Rhopalocephalus carcinomatosus," 'Centralbl. f. Bakteriologie,' vol. xiii, p. 373.

[4] Pfeiffer, 'Die Protozoen als Krankheitserreger,' Jena, Fischer, 1891, Bd. ii, pp. 202—208.

[5] Ruffer and Walker, "On some Parasitic Protozoa found in Cancerous Tumours," 'Brit. Med. Journ.;' 'Journ. of Pathol. and Bacteriol.,' Oct., 1892.

[6] Foà, "Sui parassiti e sulla patologica del cancro," 'Arch. p. le Scienze mediche,' vol. xvii, p. 253.

[7] Sanfelice, "Sull' azione patogena dei blastomiceti come contributo alla eziologia dei tumori maligni," 'Il Policlinico,' 1895.

[8] Roncali, "Sopra particolari parassiti rinvenuti in un adeno-carcinomà (papilloma infettante) della ghiandola ovarica," 'Il Policlinico,' 1895.

in diverse cases of sarcoma. It is not unlikely that the blastomycetes met with in some neoplasms are saprophytes which have accidentally penetrated into tumours already developed.

Many pathologists, with Cornil[1] at their head, deny altogether the parasitic origin of tumours, and maintain that all the cellular inclusions which up to now have been described in neoplasms are the expression of degenerative conditions of the nuclei, the nucleoli, or the cellular protoplasm. Therefore the question of the etiology of tumours still remains unsolved.

The only positive result of the studies made up till now is the particular importance of the pathological proliferation in the development of the morbid tissues. The discovery of mitoses has extraordinary importance in judging the mode of growth of a neoplasm. The frequency of mitoses in the different points precisely indicates where the development is most active: the number of the mitoses gives us an idea of the rapidity with which the growth of the tumour takes place.

But this is not sufficient: mitoses are also found in the thrombi formed by the neoplasm when it invades the blood-vessels. The thrombi, as a rule, do not contain any part of the stroma of the neoplasm, but are formed exclusively of the parenchymal cells united into plugs or disseminated in the midst of the fibrin. Nuclei in mitoses are frequently observed within these cells, which demonstrates how the neoplastic cells spreading by the embolic way reproduce the tumour, having in themselves the tendency to indefinite proliferation. The old observation of Langenbeck[2] and Waldeyer,[3] that the formation of the secondary nodules in distant parts always occurs by *metastasis*, that is by the

[1] Cornil, "Des modifications des noyaux et des cellules cancéreuses qui peuvent en imposer pour des parasites," 'Atti XI Congresso medico internaz. di Roma,' Aprile, 1894.

[2] Langenbeck, "Entstehung der Venenkrebse und die Mögligkeit Carcinome von Menschen auf Thiere zu übertragen," 'Schmit's Jahrb.,' vol. xxv, 1840.

[3] Waldeyer, 'Volkmann's Hefte,' No. 33, 1872.

transplantation of the elements of the primary focus, has been confirmed by innumerable authors.[1]

The daughter-cells of a tumour may easily penetrate into the lymph-channels, and thus reach the lymphatic glands of the nearest stations, causing an invasion of the glands themselves. We possess numerous preparations where the cancerous plugs are seen extending along the lymphatics to the nearest glands. We also have preparations where the elements of a sarcoma can be well seen within the lumen of the blood-vessels.

The most common channel by which cancerous tumours are transplanted is that of the lymphatics. In consequence of the active pathological proliferation of the cells the malignant tumour tends to expand; its elements form plugs which insinuate themselves into the neighbouring tissue, following the interstices and the lacunæ, so that they easily reach the lymph-channels.

Owing to its rapid growth the morbid tissue compresses the surrounding healthy tissues, which gradually become atrophied. Thus the morbid tissue by degrees substitutes the healthy tissue. In this way it may happen that the tumour also reaches the blood-vessels; it then compresses them, producing thrombosis, or attacks their coats, and after a variable resistance of the media, succeeds in penetrating into the lumen, and may thus give rise to infecting emboli. Owing to the resistance of the walls of the arteries this channel of diffusion of neoplasms is less frequent than that of the lymphatics. The lax connective tissue, as is readily understood, offers little resistance; and inasmuch as the interstices of the connective tissue represent the origin of the lymphatic vessels, one understands the ready diffusion of tumours by this channel. Having once reached the glands, which, as is known, act as a filter, the neoplastic

[1] Birch-Hirschfeld, "Zur Casuistick der Geschwülstembolie," 'Archiv f. Heilkunde,' x, 1869; Geissler, "Uebertragbarkeit des Carcinoms," 'Langenbeck's Archiv,' vol. xlvi, 1893; Just, 'Ueber d. Verbreit. d. melanotischen Geschw. in Limphgefässsystem,' Strassburg, 1888; Zahn, 'Ueber Geschwülstmetastasen durch Capillarembolie' u. ü. Falleseltener Geschwülstmet.," 'Virch. Arch.,' cxvii, p. 1; Zenker, "Lehre v. d. Metastasenbildung der Sarcome," 'Virch. Archiv,' cxx, 1890, p. 68, etc.

elements pause, but continue to proliferate, as can easily be seen, meeting with the karyokineses always numerous in the cancerous plugs that have arrived in the lymph-glands. Thus at length the plugs reach the efferent vessels, and from one gland pass to another, and give rise to the final metastases. The malignant epitheliomata generally follow this channel; however, the epitheliomata which have started in the abdominal organs frequently spread by the path of the veins, inasmuch as they penetrate into the branches of the vena portæ, and so produce metastatic nodules in the liver.

The veins present much more favourable conditions to the invasion than the arteries, for their coats are much less resistent. The penetration of the neoplastic tissue into a vein may sometimes give rise to an extensive thrombosis, which for a little time checks its final diffusion; but it may happen that the elements of the tumour become detached before the formation of the thrombus, and are carried by the blood-current to distant organs. In other cases it may also happen that the neoplastic tissue grows in the thrombus, and that afterwards fragments of the thrombus containing particles of the morbid tissue are detached and form infecting emboli. Not rarely individual elements or groups of elements penetrate into the capillaries, and so are carried to distant organs, producing multiple metastases. Thus it occurs, for example, that melanotic sarcomata of the eye give rise to multiple metastatic nodules in the liver, and that decidual sarcomata of the uterus frequently produce multiple metastases of the lungs.

In rare cases a tumour gives rise to numerous metastases in all the organs of the body; such cases are verified, for example, in regard to sarcomata of the lung and of the pleura, which open into a pulmonary vein, and thus rapidly reach the general circulation, by means of which the neoplastic elements are carried to and disseminated in the most distant parts.

Numerous experiments made in these latter years on epitheliomata of animals have demonstrated that such tumours are inoculable from one individual to another of the same species; that is, they are obtained by metastases from one animal to another.

Also, in the course of surgical operations on man, performed for the purpose of extirpating a malignant tumour, it has occurred sometimes that particles of the tumour involuntarily transported by the surgical instruments to other distant parts of the body have caused the development of a secondary tumour of the same nature.

CHAPTER II.

THE DOCTRINE OF PATHOGENIC MICROBES: ITS INFLUENCE ON THE PATHOLOGY OF MAN.

THE doctrine of pathogenic microbes was founded by Agostino Bassi,[1] a Lodigian physician, born in 1773.

The parasitic theory had certainly been foreseen before Bassi's time, but inasmuch as a positive basis was always wanting to such doctrine, some authors admitted it as a daring supposition, and many others positively denied it. Bassi was the first who, demonstrating in a precise way the parasitic nature of the muscardine of silkworms, gave a scientific foundation to the old hypothesis of animal contagion. He who reads Bassi's work, not published till 1835, will at once be convinced not only that Bassi was a precursor of Pasteur and Koch, but that he also was the true founder of the entire doctrine, which has waited so many years to enter the domain of science.

Bassi, after having demonstrated the parasitic nature of muscardine, after having exactly described the vegetative and reproductive forms of the cryptogam to which Balsamo-Crivelli gave the name of *Botrytis Bassiana*, invented the method of inoculations to test the pathogenic power of the micro-organism in question; and he also had an idea of the method of fractional cultures tried by him on a large scale. Bassi, following a logical concept, but which still underwent much labour to become established, founded the principle of the parasiticidal treatment, and determined the disinfecting power of various substances, using a method of experimentation even to-day universally accepted. Nor did Bassi limit himself to this, since he demonstrated the possi-

[1] A. Bassi, 'Del mal del segno, calcino, moscardino,' Lodi, Orcesi, 1835.

bility of the diffusion of muscardine by the atmospheric air, and studied the diverse paths of transmission of the contagion. He also proceeded to analyse the occasional causes of muscardine, thus anticipating by more than half a century the studies of our day. He likewise recognised in a precise way the principle of the attenuation of germs and of the exaltation of their virulence, a principle which only now is beginning to acquire direct importance in the pathology of infection. With justice, therefore, Calandruccio[1] writes in his splendid, erudite, and courageous memoir vindicating Bassi's discoveries, that Bassi has been for the parasitic theory what Darwin was for that of descent.

Bassi himself understood the full importance of his wonderful discovery, even in respect to human pathology, and, indeed, at page 9 of his work on silkworm disease, in 1835, he wrote, "This production of mine ought to interest not only the breeder of silkworms, but also all the cultivators of the natural sciences, it being capable, perhaps, of removing some of the many anomalies which the doctrine of contagions in general presents, and shedding new light,—of announcing, perhaps, the dawn of new discoveries on an argument so important and still so obscure." And in another publication Bassi wrote, "Not only am I of the opinion that the contagions, such as variola, petechia, pest, syphilis, etc., are produced by vegetable or animal parasitic entities, but also that many, not to say all, of the cutaneous diseases are due to the same cause,—that is, that these also are generated and maintained by the said vegetable or animal parasitic entities of different species; and I am also of the opinion that *some ulcers, though deep,* if they are not caused, are at least maintained, and for a long time, by parasitic entities, and that even gangrene is caused by such entities."

Bassi arrived at this doctrine not by simple theoretic considerations, but by the methodic comparison of the various human contagions with the silkworm disease, and by the study of the favourable action which parasiticides exercise on human infections as well as on the silkworm infection. Thus, for example, he recognised that "gonorrhœa is also

[1] Calandruccio, 'Agostino Bassi da Lodi, il fondatore della teoria parasitaria o delle cure parasiticide,' Catania, Martinez, 1892.

contagious, because it is produced by parasitic entities; . . . . . it can be quickly cured with *injections of corrosive sublimate* in the proportion of four grains to a small pound of water. . . .

"In inoculating children, and sometimes in large numbers, with vaccine pus the same needle is generally used, which may carry adherent to its point the germs of the contagious disease that the child who has been inoculated was affected with, and thus the same disease is inoculated into another child who is subsequently inoculated. . . . . . In inoculating the vaccine there is a way of preventing all these diseases mentioned, and of profiting by the sole beneficent fruit which is obtained from the inoculation in question. This can be obtained with little trouble and very small expense. It will be sufficient for the inoculator to carry with him, desiring to prepare for the operation, a small lamp, that is a tin vessel with a narrow neck, full of spirit of wine, and having in its mouth or projecting from the neck a wick, that is a roll of cotton; and having lighted this and made the first inoculation, the point of the needle which is employed is passed for three seconds, or three beats of the pulse, into the midst of the flame of the little lamp, then the second inoculation is at once made with the same needle, and so on from inoculation to inoculation till the whole series of operations are finished. Whatever may be the species of contagious germ that has become attached to the wounding needle, it is quickly destroyed by passing it into the said flame."

We might give many more quotations from Bassi, but the above are sufficient to enable us to conclude with Calandruccio[1] that to Bassi belongs the great merit of having been the first to demonstrate the parasitic nature of the contagions, of having established the fundamental lines of the entire doctrine of the pathogenic microbes; of having presaged that the parasitic diseases are many, that is to say, that not only the diseases generally held to be contagious are parasitic, but also many others; of having foretold that ulcers are maintained by parasites; of having, in fine, inaugurated that hygienic school to which now-a-days we all belong.

[1] Calandruccio, l. c., p. 4.

It, therefore, has been proved that not only the doctrine of the contagiousness of disease was gloriously founded and defended in Italy, as Sangalli has well observed, but also that the parasitic doctrine itself had its cradle in our own country.[1]

Bassi's work, however modest, did not fail to make a profound impression on the medicine of his day.

The "future discoveries" which Bassi had foreseen quickly began; but the doctrine that Bassi had so ably formulated very slowly entered into science.

In 1839 Schönlein[2] discovered the fungus of tinea favosa, to which Remak[3] gave the name of *Anchorion Schönleinii* in 1845, after having demonstrated that it is inoculable with success from man to man, and that thus the disease can be reproduced.

The doctrine of living contagion, for which the Italians had so obstinately combated, then barely began to make way in Germany, and Henle,[4] in 1840, accepting Bassi's doctrine and making it his own, developed all the theoretical reasons that spoke in favour of the living nature of the miasms and the contagions.

A little later Gruby[5] discovered the parasite of herpes tonsurans, and described it in 1844. These few discoveries were sufficient to open the eyes of many enlightened clinicians, and we find that in 1847 Semmelweis developed the theory of the contagion of puerperal fever, and exactly

[1] Sangalli, "Cellule e parasiti in patologia," 'Rend. del R. Istituto Lomb.,' fasc. xv, p. 673, 1884.

[2] Schönlein, "Zur Pathogenie des Impetigines," 'Müller's Archiv,' 1839, p. 82.

[3] Remak, 'Diagnost. und pathologische Untersuchungen,' Berlin, 1845–6.

[4] Henle, 'Pathologische Untersuchungen,' 1840. Hüppe ('Die Methoden der Bakterien-Firschung,' Wiesbaden, 1891) says that Henle was the first who maintained that the typical course of the infections depends upon the typical development of the parasites. This is not quite correct. He who compares Henle's work with that of Bassi will readily convince himself that the latter has preceded the former, not only in the data but also in the theory, and that Henle himself refers to Bassi.

[5] Gruby, "Recherches sur les plantes cryptogames qui constituent la maladie contagieuse du cuir chévelu décrite sous les noms de teigne tondante, Herpes tonsurans," 'Comptes rendus de l'Acad. Franç.,' 1844, p. 583.

recognised the means necessary for preventing the infection. The able intuitions of Semmelweis, however, did not find a soil prepared to receive them; his doctrine was believed to be erroneous, and he suffered a long scientific persecution for the same.

The first direct demonstration of a specific pathogenic microbe of a general infective disease belongs to Davaine,[1] who, together with Rayer, observed non-motile and very refractile filaments in the blood of a sheep, dead from anthrax. At first these authors gave little importance to their discovery, but later, after Branell[2] had confirmed it, and analogous observations were published by Pollender,[3] and Delafond,[4] and after Pasteur[5] had demonstrated that fermentation is the result of the life and the organisation of the saccharomycetes, they returned to the argument. Davaine,[6] in fact, in 1863, astonished by the resemblance of the butyric vibrio of Pasteur to the rodlets observed by him in the blood of animals suffering from anthrax, thought that as the former were the cause of the butyric fermentation, the latter might be the cause of the anthrax infection. He then made a series of experiments by transmitting the disease from animal to animal, and was able to verify that the disease is transmissible, inoculating the blood containing the bacilli, while it was not communicated with the blood deprived of these elements. He also thought that if the rodlets observed were living entities, they must very quickly multiply, and reproduce the disease even if inoculated in very small numbers; indeed, he obtained this result, injecting the blood of anthrax animals diluted a million times.

It was, therefere, very probable that the bacilli observed

[1] Davaine and Rayer, 'Bulletin de la Société de Biologie,' 1850.

[2] Branell, "Versuche und Untersuchungen betreffend den Milzbrand des Menschen und der Thiere," 'Virchow's Arch.,' Bd. xi, 1857.

[3] Pollender, 'Casper's Viertelsjahrschrift für gerichtliche und öppentliche Medicin,' Bd. viii, S. 103, 1855.

[4] Delafond, "Recueil de médecine vétérinaire," 'Repertorium für Thierheilkunde von Hering,' vol. xxii, p. 31.

[5] Pasteur, "Mémoire sur la fermentation," 'Comptes rendus,' 1857.

[6] Davaine, "Recherches sur les infusoires du sang dans la maladie connue sous le nom de sang de rate," 'Compt. r. de l'Acad. des Sciences,' 1863, tom. lvii.

were the cause of the disease. To confirm this hypothesis he endeavoured to demonstrate the constancy of the bacilli in all the cases of anthrax infection observed by him, and he also met with them in malignant pustule of man.[1]

Davaine's discovery encountered much opposition from the beginning: some authors denied the constancy of the bacilli in the blood of animals suffering from anthrax; others, such as Tigri,[2] Signol,[3] Calvert,[4] etc., affirmed that similar bacilli were found in individuals dead from the most varied diseases. Leplat and Jaillard[5] especially maintained that Davaine's bacilli were an accidental condition, because a fatal disease could be transmitted to rabbits, inoculating them with putrefied blood without bacilli. Davaine[6] and Pasteur[7] replied, dwelling on the differences which exist between the toxic agent of anthrax and that of putrefaction. Nevertheless this question did not yet appear to be solved, inasmuch as many maintained that the bacilli were an epiphenomenon, or perhaps crystals. Even the discovery of the spirilla of relapsing fever, which Obermaier[8] made known in 1873, was not sufficiently persuasive; it was not until 1877, after Koch[9] had followed the development of the anthrax bacilli, and had recognised the spores and followed their multiplication under the microscope, that the specific nature of the anthrax bacilli

[1] Davaine et Raimbert, "Sur la présence des bactéries dans la pustule maligne chez l'homme," 'Comptes rendus,' tome lix, 1894, p. 429.

[2] Tigri, divers notes in the 'Comptes rendus,' 1894; and 'Rendiconti dell' Accad. dei Lincei,' vol. xvii.

[3] Signol, "Présence des bactéries dans le sang," 'Gazette des Hôpitaux,' 1863, p. 386.

[4] Calvert, "Note rel. à la génér. spontan.," etc., 'Gazette des Hôp.,' 1864, p. 350.

[5] Leplat et Jaillard, "Note sur sujet d'expériences prouvant que le charbon de la vache, inoculé aux lapins, les tue avec tous les phénomènes du sang de rate, sans que leur sang contienne aucune trace des bactéries," 'Compt. rend.,' tom. lxi, p. 298, 1865.

[6] Davaine, "Recherches sur une maladie septique de la vache regardée comme de nature charbonneuse," 'Compt. rend.,' tom. lxi, pp. 368—523, 1865.

[7] Pasteur, "Observations verbales à la suite de la communication de M. Davaine," 'Compt. rend.,' tom. lxi.

[8] Obermaier, "Sich bewegende Fäden im Blute Recurrenskranken," 'Centralbl. f. d. med. Wissenschaften,' 1873, fasc. 10, p. 145.

[9] Koch, "Die Aetiologie der Milzbrand-Krankheit," 'Cohn's Beiträge,' 1877.

was definitely demonstrated. On the other hand, Pasteur succeeded in cultivating *en masse* the anthrax bacilli external to the organism in neutral or slightly alkaline urine. Passing a drop of the culture into new test-tubes of sterile urine, he was in this way able to obtain twelve successive cultivations of the anthrax bacilli, and he found that the liquid of the twelfth culture was quite as virulent as that of the first. By this means it was demonstrated that the anthrax bacilli isolated outside the organism, and cultivated for many generations, freed consequently from every trace of the primary material contained in the infected organism, were still capable of reproducing the disease.

Thus the method for the study of the living contagions being created, the consequences had no longer to wait.

Pasteur[1] himself took up the study of septicæmia, whose infective nature became evident through the experiments made by Signol, Calvert, Leplat, and Jaillard. Inoculating putrid blood, he obtained another disease very distinct from anthrax, due to a motile and anaërobic bacillus which he held to be the specific organism of septicæmia. Nevertheless he very prudently refrained from generalising his discovery. He, in fact, immediately admitted that septicæmia in the living is not a single disease, but that, on the contrary, there are diverse forms of septicæmia and of putrid infections, each one of which must be caused by special micro-organisms. The septic vibrio of Pasteur was later on identified with the bacillus of malignant œdema studied by Koch, and owing to the work of various observers its pathogenetic importance was recognised in respect to certain forms of infection in man.

Meanwhile, in Italy, Perrocinto[2] studied the so-called chicken cholera, and found a specific micro-organism in this disease, which was quickly confirmed by Toussaint and Pasteur.[3] The latter not only obtained the culture of Perrocinto's micro-organisms, but also ascertained that the micro-

[1] Pasteur, 'Bulletin de l'Académie de Médecine,' 1877.

[2] Perrocinto, "Epizoozia tifoide nei gallinacei," 'Ann. dell' Accadem. d' Agricoltura di Torino,' 1878.

[3] Pasteur, "Sur les maladies virulentes, et en particulier sur le cholera des poules," 'Comptes rendus,' 1880, vols. xc and xci.

organisms in the culture were greatly attenuated, and that the attenuated cultures, which no longer killed the animals, conferred on them an immunity against direct contagion and against virulent cultures, like vaccine against variola. The scientific explanation of Jenner's discovery was thus found; and such explanation was the key for discovering the methods of immunisation and of treatment of many infective diseases.

At that time, under the influence of the above-mentioned studies, a great number of investigators proceeded to search for pathogenic micro-organisms in diverse diseases, and accepted the parasitic doctrine with excessive enthusiasm, frequently supplying with their imagination the insufficiency of the methods of research. Thus, for example, Hallier[1] affirmed that the different micro-parasites were nothing more than special forms of the growth of moulds, determined by external vital conditions; these produced every sort of disease, or in certain special conditions the corresponding moulds could easily be obtained. It is needless to say that Hallier's researches were without foundation. He had attributed cholera nostras to the *Penicillium crustaceum*, Asiatic cholera to the *Urocystis orizæ*, smallpox to the *Torula rubescens*, but with these cultures of his he never succeeded in reproducing a disease; he thus completely lost himself in the mazes of an unconsidered polymorphism. A reaction to this period of fantastic exaggeration could not fail to be felt. De Barry,[2] an able botanist, immediately demonstrated that Hallier's results were worthless, because they were obtained by coarse observations, made without any precautions against the introduction of foreign germs. Under the blows of the stringent criticisms of De Barry the edifice raised by Hallier crumbled, and at the same time everything in relation to the parasitic doctrine fell into great discredit. Thus it happened that even the exact observations of many pathological anatomists, who found micro-organisms in various

[1] Hallier, "Die pflanzlichen Parasiten des menschlichen Körpers für Aerzte, Botanicker, und Studirende zugleich als Einleitung in das Studium der niederen Organismen,' Leipzig, 1866.

[2] De Barry, 'Botanische Zeitung,' 1868.

infective diseases, were not sufficient to re-establish the lost faith. Pacini,[1] in 1849, discovered bacilli in cadavers and in the dejections of persons suffering from cholera. Rindfleisch,[2] Recklinghausen,[3] Waldeyer,[4] verified the presence of micro-organisms in pyæmic processes; Sangalli[5] found them in glanders, Huter[6] and Orth[7] met with them in erysipelas, Oertel[8] in diphtheria, Eberth in typhoid, Klebs[9] in pneumonia and in other diseases; but owing to the experiments of Coze and Feltz,[10] Huter, Klebs, etc., the general doctrine did not make any progress, and even in 1880 very few believed in microbes as the cause of disease, and less still was it understood that an intimate connection existed between the new doctrines and the old, between the parasitic theory and the cellular theory.

We cannot here repeat the whole history of the long conflict, because it would exceed the limits of our theme; its history, besides, is described with much care, excepting some oversights in regard to Italian literature, in Löffler's[11] work 'On the Development of the Bacteriological Doctrine.'

Bassi's fundamental idea, developed by Pasteur with his studies on fermentation and on anthrax, was not accepted by science till after Koch's[12] researches on infective diseases of animals were published. Koch's work was possible only

[1] Pacini, "Osservazioni microscopiche e deduzioni patologiche sul cholera," 'Gazzetta medica di Firenze,' 1854.

[2] Rindfleisch, 'Lehrbuch der patologische Gewebelehre,' 1866.

[3] Recklinghausen, 'Verhandl. Anatom. der Würb. phys.-med. Gesellsch.,' 1871.

[4] Waldeyer, "Zur pathol. Anatom. der Wundinf.," 'Virchow's Archiv,' vol. xl, 1867.

[5] Sangalli, 'La scienza e la pratica della Anat. patol.,' 1.

[6] Huter, 'Allgemeine Chirurgie,' Leipzig, 1873.

[7] Orth, 'Virchow's Arch.,' 1874.

[8] Oertel, 'Zur Aetiologie der Infectionskrankheiten,' 1881. For the history see Löffler, 'Die geschichtliche Entwickelung der Lehre von den Bacterien,' Leipzig, 1887.

[9] Klebs, 'Arch. für experimentelle Pathologie und Pharm.,' Leipzig, 1873-5-6-9.

[10] Coze et Feltz, 'Comptes rendus de l'Acad. des Sciences,' 1873.

[11] Löffler, l. c.

[12] Koch, 'Untersuchungen über die Aetiologie der Wundinfektionskrankheiten,' Leipzig, W. Vogel, 1878.

through the improvement of the *technique;* that is to say, after the introduction of the microscope with immersion objectives (invented by Amici and improved by Stephenson), and the use of aniline dyes, introduced by Weigert[1] in 1875, and by Salomonsen[2] for demonstrating the bacteria in the tissues.

Koch rendered the use of the immersion lens, the staining with anilines, and Abbe's illuminator systematic and general for the quest of bacteria; finally he introduced a new improvement in the *technique* of the cultures.

This improvement consists in the use of transparent gelatine for cultivating the bacteria, as Vittadini[3] had already done for cultivating the *Botrytis Bassiana* and other Mucorinæ. Owing to this new method Gaffky[4] was able to cultivate the typhoid bacillus, and Löffler[5] that of diphtheria and of glanders.

On the same principle—that is to say, using transparent solid media, prepared by a special method (solidified blood-serum)—Koch, in 1882, succeeded in proving the infective nature of tuberculosis. The transmissibility of this disease had already been recognised in France by Villemin, and in Italy it had been demonstrated, in 1867, by the work of Verga and Biffi,[6] and of Mantegezza; Koch had the merit of discovering the specific micro-organism.[7]

Finally Koch introduced the method of disseminated cultures on gelatine; with this method he succeeded in isolating

[1] Weigert, "Ueber eine Mycose bei einem neugeborenen Kinde," 'Jahresbericht der schles. Gesellschaft f. vaterland. Cultur,' Breslau, 1876.

[2] C. J. Salomonsen, 'Studier over bloodlets Forradneln,' Copenhagen, 1877.

[3] Carlo Vittadini, "Della natura del calcino o mal del segno," 'Memorie dell' Istituto Lombardo,' vol. iii, p. 447, Milano, 1852.

[4] Gaffky, "Zur Aetiologie des Abdominaltyphus," 'Mittheilungen,' Bd. ii.

[5] Löffler, "Untersuchungen über die Bedeutung," etc., 'Mittheilungen a. d. k. Ger.,' Bd. ii.

[6] Verga e Biffi, "Sulla inoculabilità del tuberculose," 'Rendiconti del R. Istituto Lombardo,' 1867; see also Annanni, "Sulla specificità e virulenza delle sostanze caseose del Tubercolose," 'Il movimento in chirurgico,' Napoli, 1872.

Koch, "Die Aetiologie der Tuberculose," 'Berlin. klinische Woch.,' No. 15, 1882.

the micro-organisms of Asiatic cholera,[1] searched for in vain with the method of broth-culture. For this reason De Barry called the method of disseminated cultures the Columbus's egg of this phase of bacteriological *technique*.

These discoveries, owing to the precision of the methods indicated, were immediately subjected to revision and counter-proof by innumerable observers, and they were generally confirmed. The influence of these studies on pathology has been extraordinary; science, revealing the unknown agent of the contagions to our vision in the form of organised entities, began to satisfy one of its supreme aspirations, *the knowledge of causes*.

The individuality of the enemy being once known, the pathologist has been able to isolate it and compel it to live in his test-tubes, and to keep it there to determine the influences that favour it, the substances which poison it; in one word, all the conditions that are capable of exalting, destroying, or modifying its activity.

While one phalanx of observers attended to these researches on the known species, another devoted itself to the quest of the unknown agents of other infective diseases, maintaining that all were determined by bacterial species.

Koch, to demonstrate that a given micro-organism is not an epiphenomenon, but is positively the cause of a particular disease, has established three fundamental criteria; they are—

(1) The demonstration of the constant presence of that given bacterium in every case of the disease in question.

(2) The pure culture of the same for repeated generations.

(3) The reproduction of the disease in animals, by means of the injection of a culture several times removed.

With these criteria suppurations, pneumonia, rhinoscleroma, tetanus, endocarditis, and other diseases of man, of which we shall speak particularly in another chapter, were studied. Among the diseases of animals the bacterial origin of symptomatic anthrax, of cattle plague, of mastitis in cows, of pseudo-tuberculosis in rodents, of pus in bees, etc., was recognised.

[1] Koch, "Zur Erörterung der Cholerafrage," 'Deutsch. med. Woch.,' No. 32, 1884.

But for some diseases the quest for the supposed specific bacteria has been completely negative.

In malaria, for example, the supposed discovery of a particular bacillus was demonstrated erroneous by Baccelli in 1880, and, on the other hand, through the labours of Laveran,[1] of Marchiafava and Celli, and of Golgi, a new micro-organism became known, which has nothing in common with bacteria. Laveran, in 1880, described in the blood of those suffering from malaria a special organism, very curious in its optical appearances, without any resemblance, without even the most remote analogy, to any parasite whatsoever up to then known. Laveran, however, in describing a congeries of pigmented forms in the circulating blood of malarial individuals, did not furnish any proof that these forms were really living entities. Very few observers thought that Laveran's studies merited the researches of counter-proof, and Cornil, a countryman of Laveran's, maintained that the affirmations of the latter were due to an error of observation. Laveran's discovery certainly would have been lost if Marchiafava and Celli[2] had not taken it up. The two Roman observers confirmed in great part Laveran's observations; they described more minutely the small non-pigmented parasitic forms, they studied the amœboid movements, and judged them as the most characteristic forms of the malarial infection. Nevertheless criticisms and objections were not wanting against them. The forms which they had described were not found to be analogous with any known parasite; they were not shown to have a cycle of development comparable to that of a living entity; they therefore encountered the scepticism of the most authoritative observers, who, while maintaining as exact the phenomena observed by the Roman school, still interpreted them as degenerative products of the red corpuscles of the blood.

But in November, 1885, appeared a masterly work by Golgi, which opened a new horizon to pathology.

Marchiafava and Celli, as well as Laveran, had described

[1] Laveran, "Communications relatives aux parasites du sang dans le paludisme," 'Acad. de Médecine,' Nov. et Dec., 1880.

[2] Marchiafava e Celli, "Nuove ricerche sulla infezione malarica," 'Annali d' agricoltura,' Roma, and 'Fortschritte der Medicin,' 1885.

a congeries of characteristic forms in malarial blood, but they had not been able to discover anything as to their succession, to their relations with the various types of fever. Golgi,[1] with profound intuition, with the finest sagacity created by the long habitude of observing microscopic matters, succeeded in discovering the gradual succession of the diverse malarial forms, and was able to prove that the bodies discovered by Laveran feed, grow within the red corpuscles, and reproduce themselves in constant periods corresponding with the periodic succession of the febrile accesses.

The able observations of Golgi, demonstrating that the characteristic bodies of malaria feed, grow, and reproduce themselves, removed all doubt; those bodies were living entities.

Golgi's first researches dealt with quartan fever; later they were extended to tertian,[2] to the fevers with long intervals [3]—to the æstivo-autumnal fevers.

From a subtle analysis of the facts observed Golgi came to the conclusion that various sub-species or species of malarial parasites exist, species very distinct in their morphological characters and in their mode of development, which was in relation with the reproduction corresponding to the initiation of the access.

By the study of these laws that govern the development of the malarial parasites—laws discovered by Golgi—many difficult problems on the pathology of malaria were definitely solved. Melanæmia was easily explained; furthermore, it was followed step by step in its genesis, observing how the intra-corpuscular parasites gradually ate up the hæmoglobin; the granules of melanin are only the excrement, the remains of the intra-cellular parasitic digestion.

The periodicity of malarial fevers had remained a mystery from the most ancient times to our day. The most powerful intellects had abandoned the question without even formu-

[1] Golgi, "Sull' infezione malarica," 'Accad. di Med. di Torino,' November, 1885.

[2] Golgi, "Ancora dell' infezione malarica," 'Gazz. degli l' esped.,' 1886.

[3] Golgi, 'Sulla febbri a lungo intervallo,' Società med. di Pavia, Maggio, 1889.

lating its terms ; or if some one had endeavoured to indicate it, after having emitted an hypothesis, he stopped short, fearful of the darkness. Werlhof has written, "*Typorum et periodorum febrilium miracula vidit omnis ætas et obstupuit, videbit omnis posteritas forsan omnis obstupescit.*"

Golgi's discovery of the development of the malarial amœbæ, demonstrating that the reproduction of the parasite occurs according to a cycle in determinate periods, corresponding to the initiation of every febrile access, has revealed in the most simple way the mystery for so many centuries in vain attacked.

Through the laws discovered by Golgi the clinician has not only acquired precise criteria for the diagnosis of malaria, but he has also learnt to predict the accesses, to recognise the greater or less gravity of the infection, and to study the most suitable mode of administering the specific remedy.[1]

In its first period micro-parasitology was limited to making known the biological characteristics of the specific microbes of various infective diseases. But, as is readily understood, the morphological and biological study of the morbid agents outside the organism has been *only the first step* in the investigation of the general pathology of infective diseases. But the way disclosed, the first step taken, it was very natural that pathology obtained all the consequences of the new doctrine, and proceeded to discover how the virus are diffused in the environment; what the paths of introduction of the microbes into the organism are; what influence the mode of introduction of the germs and the diverse quality and quantity of the same exercise on the successive course of the disease; what the conditions are that favour the infection, and what the conditions which render it more difficult; so as to be able to observe how the virus spread and propagate in the organism, and in what mode these virus exercise their action on the living tissues.

The parasitic doctrine thus enabled pathology to solve a long series of problems in relation to infective diseases which up to then were considered unsolvable, and whose explana-

[1] Golgi, "Azione della chinina sullo svilluppo dei parassiti malarici," 'Rendiconto del R. Istituto Lombardo,' Milano, 1891.

tion was attempted by means of abstract hypotheses only. It has indicated new problems whose terms it would have previously been impossible to imagine, and it has already indicated the way for arriving at their solution.

If we wished to deal with the problems one by one, and to demonstrate step by step the particular influence which the parasitic doctrine has exercised on the pathology of each infective disease, we should be obliged to write a big treatise on the general pathology of infection. Certainly neither personal experience nor a knowledge of the literature would be wanting in us; but we should be going beyond the just proportion in the development of the prefixed theme. We shall limit ourselves therefore to the discussion of the chief general problems, and we shall thus see, in a synthetic form, what influence the parasitic doctrine has exercised on pathology.

The Paths of Transmission of the Infective Diseases.

The path of diffusion of the contagions has been the occasion of long theoretical disquisitions, in which the Italian physicians of the first half of this century gained many laurels, having foreseen with wonderful intuition the great discoveries of the future.

But the past discussions could not lead to a definite conclusion because the precise knowledge of the nature of the contagions was wanting; these consequently could not be recognised outside the organism. But since the discovery of the micro-parasites, and the determination of their specific and morphological characters, it has been possible to recognise the mode of transport of these pathogenic agents, investigating whether they exist in a given spot, whether they are absent in another, determining how they live external to the organism, how they are transmitted from one organism to another, how they succumb to the influence of definite agents.

The improved methods of *technique* have enabled Miquel,[1]

[1] Miquel, 'Des organismes vivants dans l'atmosphère,' Paris, Gauthier, 1883; and 'Annuaire de Montsouris,' *passim*.

Hesse,[1] Petri,[2] Frankland,[3] Giacosa,[4] and Roster[5] to study the *diffusion of germs in the atmosphere*, and to investigate the variations of these germs in various places at different altitudes, in diverse meteorological conditions, etc. They investigated whether the germs contained in the air were innocuous only or also pathogenic. In this way were found, for example, the streptococci of erysipelas in the air[6] of certain hospital wards (Eiselberg, Emmerich, Babes), the staphylococci pyogenes[7] in the humid air of dark habitations, and pyogenic germs were also met with in the narrow streets of crowded cities. In the wards of hospitals occupied by consumptives Cornet[8] has found tubercle bacilli, which Kustermann met with in the air of prisons.[9] But similar researches instituted on a large scale, besides having demonstrated that the air on the high ocean and that of the tops of lofty mountains does not contain a trace of any micro-organism whatever,[10] have also proved that the diffusion of germs in the open air is relatively scarce.

The pathogenic microbes pass out from the diseased organism not with the expired air, but with the excretions, the dejections, pus. All these materials fallen on floors, objects,

[1] Hesse, "Quantitative Bestimmung in der Luft enthaltenen Mikrogr.," 'Mittheil. a. d. Kais, Gesundheitsamte,' ii, Berlin, 1884.

[2] Petri, "Neue methode zur Nachweis und Bestimmung der Bacterien, etc., in der Luft," 'Zeitschrift f. Hygiene,' vol. iii, pp. 1—146, 1887.

[3] Frankland, "Studies on some New Organisms obtained from Air," 'Transact. of the Royal Society,' 1887.

[4] Giacosa, "Les corpuscles organisés de l'air des hautes montagnes," 'Arch. ital. de Biologie,' 1886.

[5] Roster, 'L' aira atmosferica,' Milano, Dumolard, 1887.

[6] Emmerich, "Nachweis von Erysipelcoccen in einem Sectionssaal," 'Tageblatt der 59 Versamml. Deutscher Naturforsch. und Aerzte,' Berlin, 1886.

[7] Uffellmann, "Luftuntersuchungen," etc., 'Arch. für Hygiene,' 1888, p. 262.

[8] Cornet, 'Die Verbreitung der Tuberkelbacillen ausserhalb des Körpers in Gefänguissen.

[9] Kustermann, "Vorkommen der Tuberkelbacillen ausserhalb des Körpers in Gefänguissen," 'Münchener med. Wochenschrift,' 1891, Nos. 44, 45, p. 789.

[10] Fischer, B., "Ergebnisse einiger auf der Plankton-Expedition ausgeführten bacteriologischen Untersuchungen," 'Zeitschr. für Hygiene,' Bd. xvii, 1894, p. 130.

linen, are immediately dangerous by direct contact only; but if such materials become dry and pulverised the dust raised in the air may transport the pathogenic germs. Thus Koch has been able to transmit tuberculosis, making animals breathe the air in a room where the dried sputa of consumptives were pulverised. Büchner[1] has communicated anthrax in the same way, thus explaining the origin of certain forms of disease in rag-pickers. Emmerich[2] has transmitted pneumonia in the same mode by the inhalation of the dust of desiccated pneumonic sputa; Bombicci[3] has thus transmitted the bacilli of influenza.

The atmosphere, therefore, presents dangers, especially in confined and badly ventilated rooms, where the seeds of contagion are found; in the open air there is such a great dilution of the pathogenic agents that they present little danger to man, especially as the germs ascend into the air adherent to the dust, and therefore tend to fall readily on the ground by the law of gravity.

The *soil* much more than the air is an immense receptacle for diverse micro-organisms. The researches of Koch,[4] Maggiora,[5] Beumer,[6] Fränkel,[7] etc., have demonstrated that the number of germs on the surface of the soil is incommensurable; but it diminishes rapidly in proportion to the depth of the subsoil. This diminution takes place in a very irregular way according to the nature of the soil, but one quickly reaches a depth, which varies according to the

[1] Büchner, "Untersuchungen über das Durchtritt von Infectionsenegem durch die intacte Lungenoberfläche," 'Arch. für Hygiene,' Bd. viii, 1888, p. 145.

[2] Emmerich und Fowitzky, 'Münchener med. Wochenschrift,' 1891.

[3] Bombicci, "Sulla diffusione dell' influenza per mezzo dell' aria," 'Riforma medica,' 1892, p. 207.

[4] Koch, 'Mittheilungen aus d. k. Gesundheitsamte,' vol. i, p. 207.

[5] Maggiora, "Ricerche quantitative sui microbi del terreno," 'Giornale dell' Accademia di Medicina di Torino,' 1887; see also Pagliani, Maggiora e Frattini, "Contributo allo studio dei microbi del terreno," 'Giornale della R. Società d' Igiene,' 1887.

[6] Beumer, "Quantitat. Bestimmungender in den Bodenproben enthaltenen Keime," 'Deutsche med. Wochenschrift,' 1886, No. 27.

[7] Fränkel, "Vorkommen von Mikroorganismen in verschiedenen Bodenchichten," 'Zeitschrift f. Hygiene,' 1887, No. 2.

places, where the earth contains no microbes. Uncultivated forests are less rich than cultivated fields; sandy soils are poorer than soils rich in humus; the subsoil of large cities still contains germs to below the level of the sewers.

The microbes ordinarily contained in the soil are amœbæ, saprophytic, nitrifying and denitrifying bacteria. All these microbes have different functions; some decompose organic substances, others fix the ammonia, and thus contribute to the purifying action of the soil. But pathogenic germs may also be found together with these; for example, in the soil of gardens the bacillus of malignant œdema, which may produce in man a form of gaseous gangrene (Koch, Hesse, Sanfelice), is nearly always found. In manured fields, on the floors of stalls, the *Bacillus tetanis*[1] is very frequently met with. After the memorable studies of Pasteur on the diffusion of anthrax in the earth it was no longer doubted that the *Bacillus anthracis* develops outside the organism, if it be not buried more than two metres in depth (Fränkel); furthermore Koch maintains that the *Bacillus anthracis* is not a strictly obligatory parasite, but a facultative parasite, which ordinarily lives in certain inauspicious soils.

But, besides its normal inhabitants, the soil of inhabited places continually receives the agents of infective diseases, together with cadavers, dejections, excretions, impure water, and sewage.

Thus it has happened that Schottelius, Gartner, and Manfredi found the tubercle bacilli in the soil; Gaffky and De Giaxa sometimes met with the cholera micro-organisms in the soil, Salomonsen the typhoid bacilli in the yard of a barrack.

Hygienists have studied what occurs to the pathogenic germs once they have reached the ground. We have already seen how many of them are raised in the air with dust, and cause infection in man.

Some, which form resistent spores, may remain viable for

[1] Sanfelice, "Contributo allo studio dei bacteri patogeni che si trovano costantemente nel terreno," 'Annali dell' Istituto d' Igiene dell' Università di Roma,' 1891, fasc. 4, p. 570; Sormani, "Tenia fecale del cetano," 'Rend. del R. Istit. Lomb.,' 1891.

a long time, until they find the suitable conditions for getting into contact with man. Others still, which are not strictly obligatory parasites, can live and multiply in the soil. In this, however, they have to sustain a struggle with the innumerable vitrifying saprophytes of the soil, and they easily succumb in the struggle for existence.

On this principle are founded the sewage farms, where the sewage, while it nourishes the soil, becomes purified of the pathogenic germs.

Among the germs which multiply in the soil, besides the anthrax bacillus, can certainly be included the typhoid bacillus and other micro-organisms, which must be the cause of infections whose etiology has not as yet been fully studied.

Thus Weil's disease and Mediterranean fever specially manifest themselves when the subsoil of large cities is disturbed, when the sewers are cleansed, or when soils loaded with decomposing materials are opened up.

Intimately connected with the diffusion of germs by means of the soil is that by *water*.

The sources of the watercourses on mountains do not contain a trace of microbes, neither do the deep subsoil waters. But the superficial springs and well water may contain them in great numbers, because they are little protected from infiltration of liquids from sewers, stalls, manure heaps, etc. Running water may collect the germs from the air, receive those from the surface of the soil, and those brought by men and animals.

The presence of germs in water having been demonstrated, and the diverse common species recognised, the numeration of the germs contained in a cubic centimetre of water taken at the source has served as the criterion for judging the purity and the *pollution* or *possible pollution* of drinking-water.

Water containing thousands of germs in every cubic centimetre (at the source) is certainly exposed to infiltrations from the surface of the soil, and consequently may from one moment to another become dangerous, since in these infiltrations the germs of infective diseases may exist,

as well as the innocuous or saprogenic germs. Large numbers of the latter in water may be in themselves dangerous to man, inasmuch as the saprophytes not only render the water less digestible, but also produce abnormal intestinal fermentations, and, as we shall see, thus pave the way for the pathogenic germs.

The pathogenic germs may reach water in various ways; not all, however, find there the conditions suitable for the conservation of their existence. Koch, in India, has discovered the cholera bacilli in the water of a pond; Nicati and Rietsch observed them in the waters of the port of Marseilles; during the late epidemic in Hamburg cholera bacilli were met with in large number in the waters of the Elbe. This river and the canals had been infected by the dejections coming from the ships in which cases of cholera were found.

Nowadays in cases of cholera epidemics the method of Schottelius is used in the quest for the cholera microbes in water; by this means a path of diffusion of the contagion may be discovered, and in this way criteria for saving many people from the infection may be furnished.

Also with regard to typhoid fever, both the Berlin and the Paris schools maintain that it is spread principally by means of water. Many authors, including Martinotti, Sonnani, etc., in Italy have observed typhoid bacilli in suspected waters. Altogether up to now sixty-five finds of the typhoid bacillus in waters have been published in literature; nevertheless we must state that absolute criteria for the differential diagnosis of the typhoid bacillus, in respect to similar bacilli existing in water, have not yet been found, as is proved by numerous recent researches. We, however, consider the opinion expressed by Monti,[1] in 1891, as justified, which, while admitting that in the present state of the science there were not sufficient criteria for diagnosing the typhoid bacillus in water, advised that water containing suspected bacilli should not be drunk.

With greater certainty other pathogenic germs are met with in water, such as the bacillus of malignant œdema, found by Pasteur in the waters of the Seine; the *Bacillus*

[1] Monti, quoted by Duclaux, 'Annali Pasteur,' 1892.

tetani, which has been observed several times in the turbid waters of some rivers.

Experimental researches have also demonstrated that the anthrax bacilli (Strauss and Dubarry), etc., can live for a long time in water.

Water is purified like the soil; the number of the germs in it is connected with the velocity of the current, the temperature of the water, the action of the contained gases, and the influence of light. Large rivers, as is now proved by innumerable studies, are rapidly purified at a short distance below the great towns situated on their banks.

But for many diseases more direct paths of contagion have been demonstrated. Uffelmann has shown that cholera germs live and multiply on dirty and moist *linen* for several days. These micro-organisms survive for twenty-four hours on dry *paper*. The microbes of suppuration may live for a long time, not only in the clothes and in the *dressings* of wounds, but also on *surgical instruments*. Cornet has demonstrated the diffusion of tubercle bacilli outside the organism, and has met with them on *furniture*, carpets, curtains, and the most varied objects contained in rooms occupied by consumptives.

Another important way for the diffusion of virus is *food* —above all, crude aliments; many fruits, and vegetables, may collect pathogenic germs from the air, from infected water, and from the contact of the fingers of diseased individuals. As Celli [1] and his pupils have shown, cholera bacilli and other micro-organisms multiply themselves on fruits; according to the researches of Gasparini,[2] tubercle bacilli live for a long time in butter Milk can transport the germs of tuberculosis, typhoid fever, and pneumonia. Meat can carry the germs of the diseases of animals transmissible to man, such as anthrax, glanders, and tuberculosis. Flies are capable of depositing various species of germs, according to Haushalter, on all kinds of food; the tubercle

[1] Celli, "Delle sostanze alimentari considerate come terreno di coltura di germi patogeni," 'Annali dell' Istitut. d' Igiene di Roma,' 1889, vol. i.

[2] Gasparini, "Il burro naturale come mezzo di trasmissione della tubercolosi," 'Giornale della Società d' Igiene,' 1890

bacilli, those of anthrax and of cholera, according to Grassi; according to Celli,[1] flies may disseminate cholera, tuberculosis, typhoid, suppuration, with their dejections. We have had occasion to see a railway employé occupied in moving waggons containing cattle, who had a malignant pustule following the bite of a gadfly. From an inquiry made it resulted that some of the animals which passed that day at the station were suffering from anthrax.

There are still other and more curious ways by which germs may spread; Fürbringer[2] found pyogenic microbes in the dirt under the nails, and thus demonstrated that Semmelweis's idea as to puerperal fever being principally transmitted by the students who went directly to the clinic from the dissecting rooms was rational.

Another mode of transmission of infectious diseases, which could be recognised only within these last years, owing to the precise knowledge we have acquired of pathogenic microbes, is the *transmission of diseases from mother to fœtus*.

This study is of capital importance to pathology, inasmuch as it has thrown some light on the problem of pathological heredity. The clinic had already demonstrated for some time that certain diseases were transmitted from mother to fœtus, but the rigorous proof of the fact was obtained by the guide of microbiology. While Davaine had maintained that the placenta was a perfect filter, capable of retaining microbes under all conditions, Perroncito,[3] in 1882, demonstrated for the first time the placental transmission of anthrax from mother to fœtus. Immediately after a similar fact was observed by Sangalli[4] and Griffini, and was subsequently confirmed by Strauss, Chamberland, Kubassof, etc. Flügge and his pupils then demonstrated that the

[1] Celli, "Trasmissibilità dei germi patogeni mediante le deiezioni delle mosche," 'Bollettino della Società Lancisiana di Roma,' 1888.

[2] Fürbringer, 'Ueber die Desinfection der Hände des Aerztes,' Wiesbaden, 1887.

[3] Perroncito, 'Sulla trasmissione del carbonchio della madre al feto,' Accad. di Med. di Torino, 1882.

[4] Sangalli, 'Bacteri del carbonchio nel feto di una giovenca morta per questa malattia,' Istituto Lomb., 1882.

passage of micro-organisms from mother to fœtus occurred when there was a lesion of the placenta (hæmorrhage). Foà and Bordoni-Uffreduzzi [1] observed the placental passage of the pneumococcus in experimental animals. Ferrari was the first to verify a similar fact in the human species. Lebedef, Hanot, and others observed the passage of the streptococcus; Ferraresi and Guarnieri demonstrated that the bacillus of glanders in the human species may also be the cause of abortion, and pass from mother to fœtus. Johne, Schmorl, and others observed the placental transmission of the tubercle bacillus; Reher, Neuhaus, Chantemesse, Vidal, Eberth, Frascani, etc., observed the passage of the typhoid bacillus from mother to fœtus. Perroncito, Lafosse, Babes, and others verified that rabies of animals also passes from mother to fœtus. These discoveries have enabled pathologists and obstetricians to make important researches on the transmission of the infections, on abortion, and on the conditions which give rise to such morbid phenomena. From the *ensemble* of these studies it results that the placental transmission of infection is impossible when the placenta is in a normal condition. But, as Resinelli [2] has observed, in the course of infective diseases there may be localisations of pathogenic micro-organisms in the substance of the placental tissue, and the lesions thus produced may give rise to abortion, without the bacilli passing into the circulatory stream of the fœtus when the detachment of the placenta does not produce rupture of the fœtal vessels. In other cases, on the contrary, when the localisation in the placenta also affects the fœtal vessels, the passage of the micro-organisms from mother to fœtus may take place; the same passage may occur in cases of infection of the blood (anthrax) by simple extravasations of the blood with the eventual rupture of the chorionic capillaries.

But besides the placental transmission of the pathogenic germs, in which there is to a certain extent a metastasis

[1] Foà e Bordoni-Uffreduzzi, "Sulla azione abortiva del meningococco," 'Rif. medica,' 1887.

[2] Resinelli, "Sur di un aborto per tifo addominale," 'Annali d' ostetrica e ginecologia,' No. 9, 1896.

from one organism to another, according to Baumgarten there is also another mode of passage from mother to fœtus,—that is to say, a *germinal transmission*. Tuberculosis, according to Baumgarten,[1] is specially transmitted by the germinative path; he, moreover, tries to exclude the mystic hypothesis of hereditary predisposition, and wishes to admit only the direct passage of the micro-organisms, not only in cases of intra-uterine infection, such as those described by Johne, but also in all the cases of the children of the tuberculous, who become tuberculous in their turn after birth. Thus the tubercle bacillus transmitted by the germinative way during conception may remain in a latent state for months, and perhaps for years. Baumgarten founds his hypothesis upon the experiments of Maffucci,[2] who, studying tuberculosis of fowls, demonstrated that when tubercle bacilli are injected into a hen's egg, this egg may still develop, but the chick which is subsequently born soon dies from tuberculosis. Such experiments, however, do not give sufficient justification for Baumgarten's hypothesis, which also admits the passage of the bacilli by means of the sperm.

Up till now the placental transmission only has been clearly demonstrated. The germinative infection always appears to be most readily admissible in certain cases of fœtal syphilis, namely, when there is a syphilitic fœtus in a healthy woman. It, nevertheless, may be said that even here the infection of the fœtus has taken place through the membranes after conception.

Be this as it may, the theory of germinative transmission is not absurd; on the contrary, it agrees with the doctrine of epigenesis developed by modern embryologists.

But even if the direct transmission of the morbid agent by the sperm or the ovum does not occur, it is very probable that the sexual products of the tuberculous are not perfectly normal. As we shall see later on, the microparasites fabricate within their host toxic substances capable of alter-

[1] Baumgarten, "Ueber experimentelle congenitale Tuberculose," 'Arbeiten aus d. Pathol. Institut zu Tübingen,' 1892, vol. i.

[2] Maffucci, "Ricerche sperimentale sull' azione dei bacilli della tubercolosi dei gallinacei e dei mammiferi nella vita embrionale ed adulta," 'Riforma medica,' 1889, Nos. 209—243.

ing the material constitution of the cells. Such toxic substances must certainly act on the ovules and the spermatozoa ; the products of conception must, therefore, be abnormal. Indeed, Hertwig, Sala, Francotte, etc., have demonstrated that if the ova or the spermatozoa are subjected to the action of poisonous substances, or to the influence of injurious physical agents, there is an abnormal development of the embryos. It is probable, then, that owing to the effect of the bacterial toxins circulating in the blood, children of tuberculous parents are born with weakened tissues, which are less resistent to the invasion of the specific bacilli, that is more *predisposed* to the disease by which the procreators were attacked.

We shall now proceed to study the predisposition and the predisposing causes that our predecessors have studied so long without finding their natural explanation.

Predisposing Causes.

The exact study of predisposing causes was not possible till after the pathogenic microbes and their specific actions were known ; consequently such study is only in its first phase, but innumerable observers are assiduously occupied with these researches, and in the course of a brief period we shall be able to substitute a positive doctrine for the indeterminate notions of our predecessors.

The clinic has demonstrated for some time that certain infective diseases occur most frequently at certain ages, in certain atmospheric conditions, in definite surroundings, and in particular conditions.

Microbiology is analysing clinical experience, and besides giving us the explanation and the precise value of the old empirical data, it has furnished us with new and exact criteria on the various predisposing conditions.

Above all, it has been demonstrated that in many cases the entrance of a micro-organism into the human body is not sufficient to produce the disease. Before the microbes which have penetrated into their host can develop at his expense, they must overcome the vital competition of the organic elements. If the microbes be very few in number

they may easily succumb, as Chauveau,[1] Watson Cheyne,[2] Wyssokowicz, etc., have demonstrated; if, on the contrary, the microbes are in considerable number, they may readily overcome the resistance of the tissues. The quantity of the virus may also vary much in relation to its varied potency, to the degree of attenuation which it has undergone external to the organism. We frequently see that a small drop of anthrax blood is sufficient to kill a rabbit; if, however, we inject a completely attenuated culture, we find that two or three cubic centimetres are not sufficient to reproduce the disease.

The *path of entry*, besides the quantity of the virus, has great importance. The pyogenic staphylococci penetrated into a sudoriferous gland give rise to a painful but rapidly curable furuncle. But if they reach the pleura they will produce a very grave empyema; if they develop in the medulla of the bones they will be the cause of an osteomyelitis readily fatal; if they nest in the cardiac valves they will give rise to an ulcerative endocarditis.

An infective disease, said Duclaux, may be defined as the struggle for existence between the cells and the parasites, as we shall see later when treating of the pathogenesis. All the influences which favour the action of the latter or weaken the activity of the former are conditions that predispose to disease. Such conditions are extremely varied, from the atmospheric agents, which change with the regions, the height, the seasons, to fatigue, hunger, traumata, poisons, and special physiological conditions.

For some time clinicians have assigned particular importance to temperature among the atmospheric influences; furthermore, they have maintained that *chill* is the sole efficient cause of pneumonia. Now we know that cold, by diminishing the activity of the elements and of the material exchange, favours the action of certain pathogenic microbes.

Thus Pasteur, Wagner, and others have observed that

[1] Chauveau, 'Comptes rendus de l'Académie des Sciences,' tome xc, p. 1526.

[2] Watson Cheyne, "Report on a Study of the Condition of Infection," 'Brit. Med. Journ.,' 1886, July 31st.

fowls are refractory to the anthrax infection, and maintain that such immunity is due to the high temperature of the body (41°, 42° C.) which birds have compared to mammifers; they succeeded in inoculating fowls chilled to 37° C. with anthrax, thus obtaining a typical anthrax infection.

Monti,[1] with the same criterion, has studied the influence of chill on the causation of pneumonia, and has demonstrated that dogs, which certainly are more resistent than man to the pneumococcus, are taken ill and die of the pneumococcic infection solely when they are inoculated after having given them a very cold bath. Thus the new doctrine of infection was reconciled with the old one of chill, and by what means the pneumococcus, which is frequently found in the mouth of the healthy, invades the lungs in determinate conditions only was explained.

Vice versâ, according to the observations of Fermi and Salsano,[2] *high temperatures* predispose to the action of other definite germs; they, for example, render guinea-pigs sensitive to the tuberculosis of fowls, against which they are ordinarily immune.

The activity of the elements of our tissues is continually maintained and rendered possible by nutrition. If the nutriment be scarce the organism becomes weakened, and presents less resistance to the pathogenic microbes. This result was demonstrated by the researches of Canalis and Morpurgo,[3] who, subjecting pigeons to long *fasting*, rendered them susceptible to anthrax, against which they are ordinarily almost completely immune.

According to the recent researches of Pernice and Alessi,[4] the deprivation of water is sufficient to produce an analogous effect.

Fatigue, by exhausting the activities of the elements,

[1] Monti, "Sulle cause predisponenti all' infezione da pneumococchi," 'Boll. della Società medica di Pavia,' 1891.

[2] Fermi e Salsano, "Sulla predisposizione alla tubercolosi," 'Riforma medica,' 1892, No. 228.

[3] Canalis and Morpurgo, "Interno all' influenza del digiuno sulla disposizione alle malattie infettive," 'Rivista d' Igiene e sanità pubblica,' 1890, Nos. 9, 10.

[4] Pernice and Alessi, "Sulla disposizione alle malattie infettive negli animali privati di acqua," 'Riforma medica,' 1891.

and producing an auto-intoxication, according to the results of the important studies of Mosso, also constitutes a condition predisposing to some infective diseases. In fact, Bouchard, Charrin, and Roger, making rats and guinea-pigs continually run in a revolving cage, saw numerous micro-organisms appear in their blood. Such an experiment is of value, inasmuch as it demonstrates the diminution of the organic resistance to the invasion of microbes. Charrin and Roger repeated the same experiment, inoculating the fatigued animals with attenuated anthrax cultures, and they saw them succumb in the space of five days, while other animals, not tired and equally inoculated, were able to survive.

Thus we can understand how the contagions attack with greater violence the poorer classes who, while continually employed in fatiguing labours, are provided for the most part with a nutriment not corresponding to their requirements, and are obliged to live without suitable protection against the atmospheric agents.

But the pathogenic microbes external to the organism are not found as in our pure cultures. They, as we have stated, are mixed with saprophytes in water, in the earth, in dirt. These saprophytes are also found in the cavities of the body communicating with the surface. They may sometimes destroy the parasites in the struggle for existence, but in contact with the organism of man they may favour their action. The saprogenic microbes, developing on decomposing organic matter, on alimentary residua accumulated in the intestine, on coagula contained in the uterus, fabricate poisons which may be absorbed, and thus exercise a debilitating influence on the organism. In this way the saprogenic microbes may open the path to the pathogenic microbes. Monti,[1] indeed, has demonstrated that in the course of the

[1] Monti, "Influenza dei veleni bacterici sulla restituzione della virulenza a microparassisti attenuati," 'Boll. Soc. med. di Pavia,' Feb. 2nd, 1889; idem, "Influenza dei prodotti tossici dei saprofiti," etc., 'Rendiconto dell' Accademia dei Lincei,' October, 1889; idem, "Sulla restituzione della virulenza ai bacilli morvosi," 'Boll. della Società medica di Pavia,' 1891; idem, 'Sulla restituzione della virulenza ai bacilli difterici,' Soc. med. Pavia, 1893.

attenuation of the pneumococcus, of the streptococcus pyogenes, of the streptococcus of erysipelas and of puerperal infection, of the staphylococci obtained from osteomyelitis and from other grave suppurations, of the bacilli of glanders and of diphtheria, a period arises in which the said microbes are no longer capable of killing experimental animals, but they may renew their virulence when the products of culture of the bacteria of putrefaction are injected with them.

Monti, therefore, concluded that his researches enabled us to explain many cases in which certain viri are for a long time preserved outside the organism, and thus renew their virulence when they come in contact with an animal. He also explained how the old authorities were right in having given importance to the accumulation of undigested materials and to intestinal disturbances, as predisposing to infective processes; as were equally right the obstetricians who had given importance to the decomposition of the lochia in the genesis of puerperal infection.

The experiments in this direction have multiplied during these latter years. Vaillard has demonstrated the influence of the pyogenic microbes on the tetanus microbes, Vincent[1] that of the pyogenic microbes on the typhoid. According to Sanarelli, the Bacterium coli increases the virulence of the typhoid bacillus; according to Löffler, the streptococci reinforce the diphtheria bacillus; according to Gautier, symptomatic anthrax re-invigorates the attenuated bacilli of hæmatic anthrax.

Following the same principle, Berinoid has shown that if large quantities of glucose be injected into animals, these animals become much more susceptible to the action of the staphylococci. This explains the frequency of boils and suppurative processes in general in the course of diabetes.

Thus we understand how some antecedent maladies weakening one or another organ or system of organs, or the whole organism, may be the predisposing cause of infective diseases.

Koch, following this order of ideas, has demonstrated that the cholera bacilli in small quantity readily die in the normal

[1] Vincent, "Études sur les résultats de l'association du streptocoque et du bacille typhique chez l'homme et les animaux," 'Annales Pasteur,' 1893, p. 141.

gastric juice; but if the hydrochloric acid be neutralised in the stomach the cholera germs pass unaltered, rapidly develop in the intestine, and produce death. This explains why, during a cholera epidemic, individuals affected with gastric catarrh, and the healthy suffering from a slight dietetic disturbance, are by preference attacked.

In the same way Lubbarsch has observed that anæmia renders individuals sensitive to diverse infections, especially those of the blood.

Mya and Sanarelli,[1] after having destroyed with hydracetin many red corpuscles in mice and pigeons, found that they were very susceptible to anthrax infection.

The recent researches of Berring, Ceni,[2] Calabrese,[3] etc., have demonstrated that the diminution of the alkalinity of the blood predisposes animals to diverse infections.

Formerly it was not understoood how *traumata* exercised a predisposing influence on infective diseases.

Above all, the traumata producing lesions of continuity, even very slight, of the epithelia of investment, may destroy the barrier opposed to the penetration of germs. But this is not sufficient; it has recently been demonstrated that traumata may determine the localisation of micro-organisms in an organ whose resistance has been partly or completely destroyed by the effect of a trauma. Thus Backer, Rosenbach, and Ollier have observed that if a small quantity of staphylococci be injected into the blood of animals, the staphylococci may succumb in the serum of the blood owing to the vital competition of the organic elements. But if a bone be broken, or a severe contusion be produced, then the micro-organisms, finding there a *locus minoris resistentiæ*, become localised, and give rise to an osteomyelitis. Traumata of the thorax may alter the contained organs,

[1] Mya e Sanarelli, "L 'azione dell' esagerata ematolisi sulla predisposizione alle malattie infettive," 'Atti dell' Acc. dei fisiocritici di Siena,' 1891, No. 10.

[2] Ceni, "Rapporti tra l' alcalinità del sangue ed il potere bactericida," 'Giorn. internaz.,' 1891.

[3] Calabrese, "Rapporti tra l' alcalinità ed il potere bactericida del siero, e loro modo di comportarsi durante l' infezione," 'Giorn. internaz. delle Scienze med.,' 1895.

producing small pulmonary lesions which permit the invasion of the pneumococci generally existing in the mouth of healthy persons. In this way Murri, Monti, and Patella explain the genesis of contusion pneumonia. The experiments made in this direction in these latter years are very numerous, and they thoroughly agree with the old clinical observations of which they have given a scientific explanation.

If we were desirous of referring to the experimental researches made with the scope of determining, in an exact way, the conditions predisposing to the diverse infections, we should have to write many pages more. It is sufficient to mention the researches on the influence that lesions of the nerves exercise on the organs (Neckan, Trambusti, Lustig); on the effect that the absence of important organs which have been extirpated or altered exercises (Canalis); on the action which poisonsing by alcohol, chloral, morphine (Platania, etc.), the inhalations of fœtid gases, the insufficiency of oxygen, the different physiological conditions, such as pregnancy (Lustig), etc., exercise. We wish particularly to mention the researches of Villa[1] on vagotomy, in which he has demonstrated that paralysis of the larynx determines the penetration into the lungs of the germs of the saliva and of the food, germs which in congestion of the lung (produced by vaso-motor paralysis) find very suitable conditions for their development.

The Pathogenesis of Infection—Toxins elaborated by Microbes.

The parasitic doctrine gave a new impulse to the study of the pathogenesis of the infective diseases, and in this field also led to unexpected triumphs.

The parasite discovered, its conditions of existence outside the organism determined, it was necessary to associate the parasites with the cells, and to establish in what mode the fundamental activities of the latter are altered by the development of the former. Two species of elementary organisms find themselves confronting one

[1] Villa, "La pneumonite da vagotomia," 'Arch. p. le scienze méd.,' 1893.

another (according to the expression of Virchow and of Brücke), the cells on the one part, the parasites on the other. Both are endowed with a life of their own, have an activity proper, dispose of forces and means proper; they come into conflict, and the consequences of such conflicts, whether they be general or local, are felt by the whole organism (transmitted by the blood and the nerves), and in their ultimate manifestations constitute the symptoms of the disease. An infective disease, therefore, has justly been interpreted as a particular case of Darwin's great law,—that is to say, as the struggle for existence between the cells and the parasites.[1]

Our fathers also thought that disease was a battle; but for them it was a mystic battle between two abstract principles—the vital force and the *ens morbi;* for us, on the contrary, it is an objective fact analysable in all its details. We are now continually circumscribing the field of battle, and determining with precision on what victory and defeat depend. To reach such a result we must endeavour to learn with the greatest possible exactness the composition and the state of the forces of both contending parties, that is of the cells and of the parasites.

With what arms do the microparasites fight? how do they produce certain special alterations of the tissues, certain functional disturbances, certain symptoms?

The answer to these problems constitutes one of the most interesting chapters of contemporary pathology. Since microbiology has enabled us to reproduce at will infection in animals, we have been able to follow the genesis of the process, its evolution, and the mechanism of its manifestations; thus we have been able to find the criteria necessary to foresee, to direct, and to combat.

Toussaint,[2] struck by the abundance of the anthrax bacilli in the blood of animals dead of hæmatic anthrax, thought that the microbes acted by simply producing capillary emboli, capable of obstructing the small vessels. But it was quickly seen that this hypothesis could not be

[1] Duclaux, 'Le microbe et la maladie,' Paris, Masson, 1886.

[2] Toussaint, "Théorie de l'action des bactéridies dans le charbon," 'Comptes rendus de l'Académie des Sciences,' Paris, tome lxxxvi, p. 978.

applied to the greater number of the infections: in fact, many microbes are never found in the blood; some of them are localised by preference in an organ or in a system, and reach the blood in the ultimate hours of the agony only. Pasteur was the first who, comparing the pathogenic microbes with the organisms of the fermentations, remembering that the latter secrete particular substances in the substrata where they develop, judged that the bacteria principally act by means of the products of their material exchange. This conception, after many contradictions, after much uncertainty, led to the demonstration of the *bacterial toxins*, which now form the argument of a new and very important branch of pathological chemistry and of general pathology.

Such studies were initiated in Italy by Selmi[1] with the analyses of the poisons contained in cadavers; Brieger,[2] in Germany, continued Selmi's work, and after him a long cohort of observers, chemists, and pathologists have extraordinarily enriched our knowledge of this difficult question.

In the present state of the science we know many products of material exchange of various bacterial species. Such products are now divided into two categories. Those of the first category result from the ordinary evolution of the bacteria, and include carbonic acid, lactic acid, fatty acids, ammoniacal substances, nitrites, nitrates, pigments, aromatic bodies, etc.

Those of the second category include the true bacterial toxins, which are at present grouped into three classes:

(*a*) Alkaloids or ptomaines;

(*b*) Toxic albumins;

(*c*) Toxic nucleins and diastases.

We cannot here write the whole history of these discoveries, and much less can we treat of the individual substances studied and of their action on the animal organism.

We shall briefly state that the ptomaines were isolated not only from cadavers, but also from cultures of the individual pathogenic microbes.

[1] Selmi, 'Sulle ptomaine ed alcaloidi cadaverici,' Accademia delle Scienze di Bologna, 1871 and later; see also in 'Berichte der deutsch. chem. Gesellschaft,' 1873–75–76–78–79–80.

[2] Brieger, 'Ueber Ptomaine,' Berlin, Hirschwald, 1885.

The study of the ptomaines of putrefaction has served as the general introduction to this kind of research. Thus were isolated parvolin, a most poisonous substance, hydrocollidin, mydalein, neurin, cholin, muscarin, a series of methylamins, butylamins, amylamins, ethylidene-diamins, whose chemical composition and toxic action on animals have been accurately studied.

From the extracts of cultures of the pathogenic microbes Brieger [1] has succeeded in isolating a series of ptomaines. Thus from the *staphylococci* and the *streptococci* cultivated on meat he isolated alkaloids endowed with slight toxic power. The staphylococci chiefly produce ammonia, the streptococci trimethylamin. We must note, however, that these micro-organisms, besides the ptomaines, elaborate other toxic substances of much greater importance,—that is to say, albuminoids and proteins. As Christmas,[2] and Brieger and Fränkel [3] have demonstrated, these albuminoids are slightly soluble in water, more readily soluble in solutions of sodium chloride, and they appear to be nearer to globulins than to the albumins of serum. From cultures of the pyogenic microbes Leber,[4] making extracts, obtained a material, chemically undefined, by which he obtained suppurations without micro-organisms. It is now generally admitted (Rodet and Courmont [5]) that the staphylococci produce multiple toxic substances, some of which are distinctly pyogenic, others act as a poison to the nervous system The streptococci, besides the acid whose chemical composition is not yet well defined, besides the ammoniacal substance, certainly produce an albuminoid poison which has positive chemotactic properties,—that is to say, is distinctly pyogenic. Manfredi and Traversa [6] have demonstrated that the cultures

[1] Brieger, "Ueber Ptomaine," 'Berliner klinische Wochenschrift,' 1886, p. 281.

[2] Christmas, Dickinck, et Holmfeld, 'Recherches expérimentales sur la suppuration,' Paris, Doin, 1888.

[3] Brieger und Fränkel, "Untersuchungen über Bacteriengifte," 'Berliner klinische Wochenschrift,' 1890, No. 11.

[4] Leber, 'Die Entstehung der Entzündung,' Leipzig, Englemann, 1891.

[5] Rodet et Courmont, "Produits du staphilocoque pyogène," 'Le Bulletin médical,' 1892, p. 84.

[6] Manfredi e Traversa, "Sull' azione fisiologica e tossica dei prodotti di

filtered through a Chamberland's bougie are toxic for animals.

The cultures of the pneumococcus also contain diverse toxic substances, including an acid which coagulates the serum when cultures are made in liquid serum. Moreover, there are at least two albuminoid substances, one of which passes in the filtrate, and was called pneumotoxin by Klemperer;[1] another, obtained by Foà,[2] making a glycerine extract of the bacteria, is called pneumoprotein.

The toxic products of the *tetanus* micro-organisms have been much studied.

Brieger, in 1886,[3] studying the impure cultures with a method not free from criticism, and which, on the contrary, according to many authorities, may cause the breaking up of the toxic substances by the normal albuminoids, obtained four different ptomaines—tetanin, tetanotoxin, spasmotoxin, and another indeterminate base. The effects, however, of these bases on the organism of animals were not certainly identical with those of tetanus, and consequently it was felt necessary to study the tetanus toxin from another direction.

Faber,[4] in 1890, was the first to show that the toxin elaborated by the tetanus bacilli is an albuminoid, which is altered by a high temperature, but which can be separated from the bacteria by filtration through a Chamberland filter. Brieger and Fränkel,[5] who took up these studies, demonstrated that the filtrate is extremely virulent, but loses this property when heated to 65° C. On drying the cultures in the exsiccator they obtained a substance which killed white rats with tetanic symptoms in a dose of three millionths of a gramme. Recently Brieger and Boer [6] have found that the

coltura degli streptococchi della risipola," 'Giornale internazionale della Sc. med.,' 1888.

[1] Klemperer, G. u. F., "Versuche über Immunisirung und Heilung bei Pneumococcen-Infection," 'Berlin. klin. Wochen.,' 1891, Nos. 34, 35.

[2] Foà e Scabia, "Sulla pneumoproteina," 'Gazzetta med. di Torino,' 1892, No. 22.

[3] Brieger, "Ueber Ptomaine," 'Berlin. klin. Wochen.,' 1886.

[4] Faber, Kund, "Om tetanus som Infectionssydom," Kjöbenhaven, Gyldendal, 1890.

[5] Brieger und Fränkel, l. c.

[6] Brieger und Boer, "Ueber Antitoxine und Toxine," 'Zeitschrift für Hygiene u. Infectionskrankheiten,' vol. xxi, p. 259.

tetanus poison can be separated from the filtrate, precipitating it with zinc chloride. The collected precipitate can be broken up with phosphate of soda. According to Tizzoni, Cattani,[1] Vincent, Vaillard, etc., this substance has the characters of a diastase. It was on the basis of these studies that bacteriologists were able to proceed to the immunisation of animals, and to give foundation to the principle of serum-therapy.

The toxin elaborated by the *diphtheria bacilli* was demonstrated for the first time by Roux and Jersin[2] by means of the filtration of the cultures, and they classified it among the enzymes. Brieger and Fränkel,[3] who confirmed the discovery of Roux, observing that the toxin resisted evaporation to a temperature of 50° C., maintained that it was a derivative of serum-albumin. These authors also isolated the toxin from the blood and the cadavers of diphtheritics. According to Freund and Grosz, the diphtheria toxin can also be precipitated with nucleinic acid; when heated for two hours at 58° C. the toxin completely loses its potency. Roux and Jersin obtained it by drying in the form of a dry powder; Brieger isolated it by the zinc chloride method already mentioned for tetanus.

This diphtheria toxin produces the same effects, local and general, on animals that are obtained by inoculating the pure living and virulent microbes.

With regard to *typhoid bacilli*, Brieger, in 1885, obtained from the cultures an alkaloid which he called typhotoxin, very poisonous for animals, and capable of producing death with symptoms of diarrhœa; but Salkowsky maintains that the typhotoxin of Brieger was an artificial product, due to the method of preparation. Later Brieger and Fränkel obtained from the cultures, by the method already mentioned for other diseases, a toxic albumin; Sanarelli,[4] adopting a similar method and using very virulent cultures, confirmed the

[1] Tizzoni e Cattani, *passim* in 'Riforma medica.'

[2] Roux et Jersin, "Contribution à l'étude de la Diphthérie," 'Annales de l'Institut Pasteur,' 1888–9.

[3] Brieger and Fränkel, l. c.

[4] Sanarelli, "Études sur la fièvre typhoïde expérimentale," 'Annales Pasteur,' 1894.

existence of the toxic albumin (which he considered analogous to the proteins), and he observed that this substance produces in animals a grave poisoning, which manifests itself not only with nervous disturbances and collapse, but also with lesions of the enteric mucosa, and especially of Peyer's patches.

According to Cesaris-Demel and Orlandi,[1] the products of the *Bacterium coli* were equivalent to the soluble products of the typhoid bacillus. Even the toxins elaborated by the *Bacterium coli* were proteins, and their action on animals was altogether identical with that of the typhoid protein. This doctrine of the equivalence of the toxic products would confirm the idea of the Lyons school, according to which the typhoid bacillus is only a variety of the *Bacterium coli*. But Marenghi,[2] for the first, has demonstrated that this equivalence cannot be admitted in an absolute way. He, in fact, found that the serum of convalescents from typhoid fever injected into rabbits retarded and sometimes annulled the effect of the typhoid bacillus. On the other hand, the same serum injected with the *Bacterium coli* powerfully increased the virulence of the latter. From these experiments it results, be it even in an indirect way, that the toxic products of the typhoid bacillus are not equivalent to those of the *B. coli*.

Pfeiffer's experiment, demonstrating the agglutinative action of typhoid serum on the typhoid bacillus (we shall describe this experiment in the next chapter), confirms Marenghi's statement, inasmuch as the typhoid serum does not agglutinate the *B. coli*. This fact, confirmed by Grüber, Löffler, Abel, and Funk, verifies that positive differences exist between the *B. coli* and the *B. typhosus*. The agglutinative substance, according to Grüber,[3] is a specific product of the typhoid bacillus.

The soluble products of the *Bacillus pyocyaneus* were accu-

[1] Cesaris-Demel ed Orlandi, "Sull' equivalenza biologica dei prodotti del Bacterium coli e del b. typhi," 'Archivio p. le Scienze mediche,' 1893, p. 279.

[2] Marenghi, 'Azione del siero di convalescenti da febbre tifoide sulla virulenza del bacillo del tifo e del B. coli,' Soc. Med. di Pavia, 1895.

[3] Grüber, "Priorität, Anspruch," etc., 'Deutsch. med. Wochen.,' 1896.

rately studied, above all, by Francesi. Gessard [1] has studied particularly the pigment, or, better, the pigments, inasmuch as it has been shown that the *Bacillus pyocyaneus* elaborates a pyocyanin, which isolated has a beautiful blue colour, a pyoxanthose, that has an intense yellow colour, and a pyofluorescein, which presents a beautiful green fluorescence.

But these coloured substances do not represent the toxin of the *Bacillus pyocyaneus*. As has been demonstrated by Charrin,[2] and confirmed by Guiquard and by Arnaud, the *Bacillus pyocyaneus*, besides the pigments, elaborates volatile products separable by distillation, which have an action on the organism, inasmuch as they diminish the excitability of the vaso-dilator apparatus. Moreover the *pyocyaneus* elaborates non-volatile products soluble in alcohol that have an elective, and especially a convulsant action on the nervous system. Finally, the *pyocyaneus* elaborates a substance, probably albuminoid, precipitable by alcohol, alterable by heat, non-dialysable, which gives rise to diarrhœa, wasting, fever, and sometimes albuminuria and hæmorrhage.

These researches on the *pyocyaneus* have greater importance than has been believed, inasmuch as Charrin,[3] Kossel,[4] Neumann, Karlisky, etc., have recently observed a disease in man, and especially in children, due to the *pyocyaneus*, and characterised by the above-described symptoms.

The earliest researches on the toxic products of the *anthrax* bacillus were made by Hoffa [5] in 1886; later Christmas, in 1888, proved that a toxic albuminoid is produced in anthrax cultures. Hankin, in 1889, isolated from the cultures an extraordinarily toxic albumose, which was afterwards met with by Brieger and Fränkel, who extracted it in the form

[1] Gessard, "De la pyocanie et de son microbe," 'Thèse de Paris,' 1882; idem, "Sur les pigments divers produits par le microbe pyocyanique," 'La Semaine médicale,' 1890, No. 9.

[2] Charrin, 'La maladie pyocyanique,' Paris, Steinbel, 1889; Amand et Charrin, 'Bulletin médical,' 1891; Charrin et Gley, 'Compt. rend. de la Soc. de Biologie,' 1892, etc.

[3] Charrin, "Maladie pyocyanique chez l'homme," 'Compt. rend. de la Soc. de Biol.,' 1890, p. 496.

[4] Kossel, Hermann, 'Zur Frage der Pathogenität des *B. pyocyaneus* f. d. Menschen," 'Zeitschrift für Hygiene,' vol. xvi, No. 2.

[5] Hoffa, 'Die Natur der Milzbrandgiftes,' Wiesbaden, Bergmann, 1886.

of a grey powder soluble in water. Hankin,[1] repeating his experiments together with Westbrook, found that the anthrax bacilli produced in the cultures a proteolytic diastase which breaks up the proteids and gives rise to a very toxic albumose, while decomposing the peptones the same diastase produces a substance immunising for animals sensitive to anthrax.

The bacillus of *malignant œdema*, according to the studies of Kerry[2] and others, breaks up the albuminoids and furnishes diverse products of putrefaction, such as fatty acids, leucin, hydroparacumaric acid, derived from valerianic acid, etc. But, according to Chauveau, Arloing, Chamberland, Roux, Besson,[3] etc., on filtering very active cultures a toxic liquid is obtained, of which 6 c.c. is sufficient to kill a guinea-pig. This soluble toxin is altered at a temperature of 80° C., becomes decomposed in time, and, according to Besson, has negative chemiotactic properties.

From the cultures of the *Bacillus tuberculosis*, as is known, Koch[4] prepared his tuberculin, extracting with glycerine the cultures *en masse* of tubercle bacilli, filtering the extract, and precipitating it by the addition of alcohol 60 per cent. The tuberculin isolated in the dry state, and then redissolved, presents all the reactions of the albuminoids, is precipitated by nitric acid, phosphomolybdic acid, acetate of iron, ammonium sulphate, and alcohol 60 per cent. It is soluble in glycerine and partly soluble in water. Tuberculin in healthy animals produces a general reaction in a large dose only; in the tuberculous it determines a local and general reaction even in small doses. On the other hand, tuberculin does not seem to be the principal toxin elaborated by the tubercle bacilli. Prudden,[5] Strauss, and Gamaleia have

[1] Hankin et Westbrook, "Sur les albumoses et les toxalbumines sécrétées par le bacille charbonneux," 'Ann. Pasteur,' 1892, vol. vi, p. 633.

[2] Kerry, "Ueber die Zersetzung des Eiweisses durch die Bacillen des malignen Oedems," 'Sitzungsberichte d. Academie der Wissensch. zu Wien,' 1889.

[3] Besson, "Contribution à l'étude du vibrion septique," 'Ann. de l'Institut Pasteur,' 1895, No. 3.

[4] Koch, "Weitere Mittheilung über das Tuberculin," 'Deutsche med. Wochen.,' 1891, p. 1189.

[5] Prudden and Hodenpyl, "Studies on the Action of Dead Bacteria in a Living Body," 'New York Med. Journ.,' 1891, June 6th—20th.

demonstrated that a tuberculous eruption can be obtained in the viscera, inoculating sterilised cultures of the tubercle bacilli. Naturally, however, the disease is not transmissible from animal to animal. With all probability the toxic bodies of the tubercle bacilli appertain to the group of the proteins.

The study of the toxic products of the *glanders* bacilli is still little advanced. It is almost limited to the preparation of the so-called mallein, which is obtained by means of glycerine extracts like those of tuberculin. Mallein, according to Nocard and Roux[1] (like tuberculin for tuberculosis), is very useful for making the early diagnosis of glanders in horses; in fact, the glandered animals react in a very special way to the injection of a small quantity of mallein. A quarter of a cubic centimetre is sufficient to produce a notable rise of temperature (from $1\frac{1}{2}^{\circ}$ to $2\frac{1}{2}^{\circ}$ C.) and a local reaction, characterised by a painful tumefaction. Healthy animals do not react even to much larger doses.

There is no doubt that the discovery of tuberculin and of mallein has given a great impulse to the study of inflammation.[2] These substances introduced into the circulation exercise an elective action on those tissues which are invaded by the specific micro-organisms, and determine an inflammatory reaction in them that sometimes gives rise to an involution of the process. Nevertheless up till to-day the reason of such elective influence has not been explained. Independently of such localisation, if tuberculin and mallein be introduced into the healthy organism in sufficient quantity they produce fever and leucocytosis. This fact, perhaps, will enable us to explain the above-mentioned local influence.

With regard to *cholera*, Cantani[3] has believed for some time that the gravest symptoms of the algid stage present the characters of an intoxication effected by substances absorbed from the intestine, and, in fact, he has demonstrated that old

[1] Nocard, 'Sur la malleine,' VIII Congress di Hygiene in Budapest, 1894.

[2] Hertwig, "Ueber die physiologische Grundlage der Tuberculinwirkung," 'Eine Theorie der Wirkungsweise bacillärer Stoffwechselproducte,' Jena, Fischer, 1891.

[3] Cantani, "Ueber die Giftigkeit der Cholerabacillen," 'Deutsche med. Wochen.,' 1886, p. 789.

cultures of the cholera bacilli filtered or sterilised have a potent toxic action on animals.

From the cultures Brieger has isolated traces of alkaloids, and among these cadaverin in abundance; Kunz also found spermin; Petri isolated a toxic peptone which he called toxo-peptone; Scholl isolated a choleraic toxoglobulin.

The successive authors, however, have demonstrated that the chemistry of cholera is much more complicated.

Hüppe,[1] considering that in the intestine the cholera bacilli live in relatively anaërobic conditions on a medium very rich in albuminoids, thought of cultivating the cholera bacilli within fresh eggs sealed with paraffin after the inoculation. By this means he obtained the abundant production of a toxic suhstance which, like Scholl, he considered a toxo-peptone, derived from the breaking up of the albumin. Pfeiffer,[2] however, thought that this substance was not the principal cholera toxin. He therefore took up the study at the point where Cantani had left it, and, like the latter, came to the conclusion that the principal toxins of cholera are integral parts of the body itself of the micro-organisms. Instead of sterilising the cultures with steam, as Cantani had done, he sterilised them with the vapour of chloroform, and he found that 10 milligrammes of culture thus sterilised were sufficient to kill a guinea-pig of 200 grammes.

Such inoculation causes profound collapse, thermic depression, clonic convulsions, and death in eight to twelve hours.

The bacterial toxin studied by Pfeiffer is very readily alterable, but in decomposing it gives rise to secondary products, which are also toxic, but in a much less degree. The primary toxin, different from the toxins of diphtheria and tetanus, acts very rapidly without incubation. It is extremely powerful when injected directly into the blood; in the peritoneum and subcutaneously it is fatal in larger doses only. In the stomach it is not absorbed when the epithe-

[1] Hüppe, "Ueber die Aetiologie und Toxikologie der Cholera asiatica," 'Deutsche med. Wochen.,' 1891, No. 53; idem, "Der Nachweis des Choleragiftes beim Menschen," 'Berliner klinische Wochen.,' 1894, p. 395.

[2] Pfeiffer, R., "Untersuchungen über das Choleragift," 'Zeitschrift für Hygiene,' vol. ix, 1892, and other papers in the same journal.

lium is perfectly intact; but if tincture of opium be injected into the peritoneum, if a slight lesion be produced in the intestine, a fatal intoxication takes place.

Pfeiffer maintains that even in man the symptoms of intoxication of the algid stage, so well analysed by Cantani, characterised by paralysis of the circulatory centres and by depression of the thermogenesis, are due to the rapid absorption of the toxic substances of the cholera vibrios. In agreement with this idea, Pfeiffer notes, as Paccini had already done, that cholera is an infective process of the intestinal epithelium, in which the vibrios insinuate themselves between the epithelia, determine their partial necrosis, and thus uncover the tissue of the mucosa, from which the bacilli may be directly absorbed. The more extensive the lesion of the mucosa the more rapid the intoxication.

This doctrine also explains very well the cases in which though the diarrhœa is very profuse, with large masses of bacilli in the fæces, still the symptoms of intoxication do not appear grave. In these cases either the epithelial destruction is wanting, or the profuse diarrhœa has prevented the concentration of the germs in the mucosa.

In agreement with the above-mentioned doctrine of Pfeiffer, Bosc[1] has demonstrated that the blood during the asphyxial period of cholera is strongly toxic for experimental animals, and injected into rabbits kills them with the same symptoms which are observed in rabbits inoculated with cultures of the cholera vibrios.

The Evolution of Infection.

The micro-organisms then attack their host principally by means of the toxins elaborated by them. At all times, during the study of the mechanism of infection, the examination of the symptoms or of the lesions that develop, and the analysis of the evolution and of the termination of the infective disease, we have to do with the toxins elaborated by the microbes.

[1] Bosc, "Des propriétés cholérigènes des humeurs des malades atteintes [de] choléra asiatique," 'Annales Pasteur,' vol. ix.

The bacterial toxins may act locally, or they may enter the circulation and exercise their influence on distant parts: altering the material exchange, they may thus diminish, exalt, or pervert the fundamental activities of the organic elements.

The organism invaded by microbes, intoxicated by their toxins, may triumph over its invaders, defending itself by its forces alone or with the aid of medicine. The means by which the organism defends itself against the parasites are to be sought in the activity proper of the elements. These elements, owing to the continuous vital competition, and the great biological law of the struggle for existence and of the adaptation to the environment, have necessarily adapted themselves to the contact of many noxious substances, and have gradually assumed the faculty of destroying and eliminating them.

We know that substances injurious or useless to the healthy organism do circulate in it,—the leucomaines of Bouchard and Gautier, which are various products of the processes of disassimilation, or transitory products in the course of the oxidation of the albuminoid substances.

Now the cells of the organism have to adapt themselves and habituate themselves to resist the action of such noxious substance, and in some way neutralise its influence.

This power of neutralising the toxins must be inherent in the living protoplasm. It must be one of the essential chemical components of the protoplasm, one of the primary products of Kossel (leuconucleochiston), that which has the power of neutralising the physiological toxins and the bacterial toxins.

Metschnikoff's researches have demonstrated that in the lower animals the cells of the mesoderm accumulate in the parts invaded by the parasites, ingest the microbes, and digest them. Disease manifests itself here as a particular case of the struggle for existence in its most direct form. The afflux of the leucocytes to the inflamed spots is therefore explained as a social function of defence; the destruction of the micro-organisms by the work of the cells has its phylogenetic explanation in the intra-cellular digestion of the inferior organisms. In this way the entire process of

inflammation finds for the first time a naturalistic interpretation.

In the higher animals, where the division of labour and the differentiation of parts are more profound, where consequently a greater co-ordination exists, the function of defence has become collective, and appertains principally to the serum of the blood and to the juices of the tissues, as was demonstrated for the first time by Fodor, Flügge, and Nuttal.

But as the serum and the organic juices are elaborated by the cells, it is readily understood that it is always the cells which organise their own defence.

Intoxicated by the bacterial secretions, the organism continues its work of defence, expelling the toxic bodies by the kidneys, transforming them in the liver, burning them up in the blood and in the tissues, elaborating substances that neutralise them.

By the analyses of all these physio-pathological processes pathology has finally succeeded in explaining the clinical symptoms, co-ordinating the study of the latter with the analyses of the histological and the histochemical alterations produced by the bacteria and by their secretions on the cells of the organism.

The histopathological and histochemical lesions produced by the microparasites vary according to the bacterial species, according to their degree of virulence, the path of entry, the diverse conditions predisposing to infection, the varying resistance of the attacked organism.

Some *saprophytic* bacteria, such as the *Bacillus subtilis*, the micrococci described by Bizzozero in the normal human epidermis, are incapable of invading the organism; even when inoculated in large quantities they are readily ingested by the leucocytes and rapidly destroyed. Interesting observations on this argument have been published by Golgi[1] in his study of the supposed *Bacillus malariæ* of Klebs, Tommassi-Crudeli, and Schiavuzzi.

Many saprophytes, however, and especially the microbes

[1] Golgi, "Sul preteso *Bacillus malariæ* di Klebs, Tommassi-Crudeli, e Schiavuzzi," 'Archivio per le Scienze mediche,' 1891.

of putrefaction, are capable of elaborating toxins of which we have already spoken, toxins which absorbed by the organism may produce grave intoxications. Such is the origin of botulism, according to the innumerable studies made by Brieger and others; such is, according to Cuzzi, the origin of the sapræmia of surgeons, in which an absorption takes place of the toxin elaborated by the saprophytes on the surface of ulcers or in endouterine coagula. In the same way Bouchard has explained certain auto-intoxications that are produced by the decomposition of fæces or imperfectly digested food in the intestine.

Other microbes, which are called *infective* or *virulent*, give rise to disease by developing within the organic tissues. These are the pathogenic microbes proper.

Such microbes develop only in the place of inoculation, but they exercise an extremely injurious influence on the organism by means of their toxins which enter the circulation. Such are, for example, the *tetanus* bacillus and the *diphtheria* bacillus.

We note, however, that, according to Frosch's researches, the diphtheria bacillus in man, besides the intoxications, may also cause a general infection passing into the blood.

The *gonococcus*, the *influenza bacteria*, the *spirilla of Asiatic cholera*, etc., have a limited development on the surface of certain mucosæ, but they also exercise a more or less violent action by means of their toxins (cholera, influenza).

Many micro-organisms give rise to diverse processes according to their seat; they may be the cause of local processes and of general diseases. Such are the pyogenic micro-organisms, the pneumococcus, the tubercle bacillus.

The *staphylococci* by their destructive action on the tissues may attack the walls of the vessels, and thus penetrate into these vessels, and produce infecting emboli; we then will have the picture of a pyæmia.

The *streptococci*, spreading by the lymphatics, give rise to lymphangites, adenites, inflammation of the serosæ; when they invade the blood they produce septicæmic forms. The general processes which occur in *pyæmia* are due principally to the multiple suppurations. The name of *septicæmia* is given essentially to the morbid forms in which the micro-

organisms from a primary focus pass into the blood; such is the case in anthrax, hæmorrhagic septicæmia, and in certain general infections from the streptococci, the pneumococci, and sometimes the diphtheria bacilli.

Typical *infections of the blood* occur in malaria and recurrent fever.

The study of the *local lesions* produced by bacteria have enabled pathology to reconstruct with great richness of detail the chapter on *inflammation*; to analyse how ordinary inflammation, the specific inflammations (according to Sangalli and Rindfleisch), or the infective granulomata (according to Virchow and Ziegler) develop. This new phase of studies has positively demonstrated that in inflammation there are not only circulatory disturbances and nutritive disturbances, but also formative disturbances and phenomena of proliferation.

The bacterial species which give rise to inflammation are diverse, and each of them, according to Bard, impresses a particular character on the process. The pyogenic microbes, the pneumococcus, the attenuated anthrax bacillus, inoculated subcutaneously, produce, above all, dilatation of the vessels, which facilitates œdema and diapedesis. It is very quickly observed that the nutrition of the tissue is altered; many elements poisoned by the bacterial toxin appear affected by a process of necrosis by coagulation.

The processes of hydration, peptonisation, digestion, explain these phenomena, and in part also the œdema; the diapedesis of the leucocytes (the greater part multinucleated) also very quickly begins.

How does this diapedesis take place? Metschnikoff's researches have indicated the phylogenetic origin of the phenomenon, but they have not explained why the leucocytes are attracted even from distant parts to the inflamed focus. This phenomenon was not explained till after the celebrated researches of Pfeiffer on *chemiotaxis* were published.

Just as legumin, casein, glycocol, and many other bodies slowly diffusing themselves in liquids attract the elementary organisms, that is are positive chemiotactics, so certain products of the pyogenic microbes, of the pneumococcus, of

Friedländer's bacillus, of the attenuated anthrax bacillus, etc., diffusing themselves in the parenchymæ and in the blood, attract the motile cells, which thus accumulate in the inflammatory focus. In this way, according to Massart, Budet, Gabritchewsky, and others, is very well explained the cause by which diapedesis takes place in inflammation.

But other substances exist, such as urea, scatol, trimethylamin, etc., which exercise a negative chemiotactic action, —that is to say, repel the lower organisms. Thus we find that the toxic products of certain very virulent bacteria repel the leucocytes of the blood.

Büchner has made a step forward in this chapter of inflammation, demonstrating that a large number of bacteria contain in their body substances capable of exercising a positive chemiotactic action. Extracting the residuum of bacterial cultures with potash ½ per cent., Büchner isolated from many species a substance, bacterioprotein, which, introduced under the skin within small tubes of glass or of celloidin, slowly diffusing itself, attracted the leucocytes in large numbers. It therefore results that the positive chemiotactic substance is contained in the body proper of the bacteria, and the suppuration occurs precisely when the micro-organisms succumb, and thus furnish the chemiotactic substance; and this is actually observed in the formation of abscesses. The septicæmic germs, like the virulent anthrax bacterium, do not produce suppuration exactly because they do not die, but develop continuously and rapidly in the organism, attacking it with their toxic products. But if the anthrax bacilli be injected into animals highly immune, such as white rats, or if attenuated cultures be injected, incapable of elaborating potent toxins, then the bacilli die in large numbers, and local suppuration takes place. In this way suppuration has no longer a specific character, inasmuch as it can be determined by diverse species of micro-organisms.

The micro-organisms, however, may give a special character to the suppuration owing to the action of their other products; thus the staphylococci rapidly decompose the fibrin and cause a genuine suppuration.

The pneumococci, on the contrary, not having any pepton-

ising power, give rise to inflammations with a distinctly fibrinous character.

The diphtheria bacilli elaborate a toxin which has a very powerful necrotising action; such action is felt, above all, in the focus, but it extends immediately to the whole organism by the entry of the toxin into the circulation.

The infective granulomata represent the products of specific inflammation, and are due to particular micro-organisms, which cause a characteristic species of granulomata, such as tubercle, leprosy, glanders, pseudo-tuberculosis, etc.

Among these granulomata *tubercle* has been particularly studied, where Baumgarten demonstrated that under the influence of the bacilli and of their products an irritation of the tissue, which conduces to the karyokinetic proliferation of the fixed elements of the connective tissue, especially occurs. Later, owing to the absence of vessels and the slow action of the tubercle toxins, necrobiosis of the tubercle takes place. The young elements derived from the fixed cells of the connective tissue constitute the epithelioid cells, which frequently appear in mitoses, and thus form the nodular foci, in the midst of which giant-cells are also found containing bacilli, necrotic in the centre and rich in numerous nuclei at the periphery. With the necrobiosis of the tubercle commences, according to Baumgarten, the diapedesis of the leucocytes, which may migrate even in large numbers, filling all the tubercle, and thus impressing on it a particular character. With the necrosis of the tissue and the diapedesis a serous exudation, and sometimes a precipitation of the fibrin, also occur. This process continuing, the caseification of the tubercle gradually takes place.

The genesis of *leprous nodules* is very similar to that of the tuberculous nodules; even here there is a characteristic vegetation of the fixed cells of the connective tissue; the bacilli are generally found within the cells, which sometimes may become gigantic, but they never assume the typical aspect of the tuberculous giant-cells. The leprous cells are much more resistent, and only when they are crowded with bacilli do they succumb and form vesicles full of micro-organisms.

The leprous nodules do not readily soften and ulcerate; they never undergo caseification; on the contrary, they frequently become slowly reabsorbed.

In *glanders* also the so-called snotty nodules are found, but they have without doubt a different histogenetic origin from the tuberculous nodules. The elements which compose them have all the characters of migrated leucocytes, which in the focus undergo a process of necrosis. Thus the softening of the nodules and the formation of ulcers occur.

For some time the clinic has demonstrated that, in the infective diseases, there are general symptoms besides the local symptoms and the local effects. But it was the development of the parasitic doctrine which enabled clinicians to distinguish the two categories of manifestations, and to analyse them in their pathogenesis. We now know that a large number of microbes can vegetate in a circumscribed organ and give rise to particular alterations in it, while the diffusion in the organism of the various toxic products determines the most severe general manifestations. To-day, then, pathologists maintain that every infection is accompanied by an intoxication.

The general symptom which occupies the first place in every grave infection is without doubt *fever*.

It is true that there are slight infections,—as, for example, small boils; or grave infections with a very chronic course,—such as certain forms of tuberculosis, which may run without fever. This must happen every time that the pathogenic microbes develop few toxic substances, which are insufficient to increase the thermogenesis. In 1889 Charrin and Ruffer were able to determine that a pure sterilised culture deprived of all living germs inoculated in a certain quantity in animals caused a rise of temperature. Roussy has given an analogous demonstration with diverse micro-organisms.

Bouchard has discovered that among the various bacterial secretions toxins are found which act without increasing the temperature, others which have a distinctly febrigenous action, others still which determine a lowering of the temperature below the normal.

Centanni,[1] from a series of cultures of streptococci, staphylococci, anthrax, typhoid, tetanus, tubercle, diphtheria, and other bacilli, separated a febrigenous substance that he called pyrotoxin. This substance inoculated in animals has always the same action, whatever be its origin, and it produces a rise of temperature of 2° or 3° C. and more. The pyrotoxin of Centanni, owing to its mode of preparation, has been considered very similar to Büchner's protein. As we have already mentioned, tuberculin and mallein are also febrigenous; they also, according to Römer, are analogous to the proteins.[2]

The bacterial toxins also frequently exercise a general action on the *blood*. As results from the clinical observations of Limbeck, Rieder, etc., in diverse forms of infection there is a leucocytosis, in others a diminution of the white corpuscles. The studies of Massart and Bordet,[3] Steinhaus, etc., on bacterial toxins show that the leucocytosis is due to the chemiotactic properties of certain bacterial secretions which, arrived in the blood, exercise an irritative action on the hæmatopoietic organs, and thus determine an abnormal proliferation of the white corpuscles.

Very notable also is the action of the micro-organisms on the *red corpuscles* of the blood. The amœbæ of malaria, as is known, develop within the red corpuscles and devour the hæmoglobin, leaving a residuum of melanin. Other micro-organisms destroy the red corpuscles by means of their toxins. Thus Fischel, Adler, and others have demonstrated that the secretions of the streptococci have a potent anæmising

[1] Centanni, "Studio sulla febbre infettiva il veleno della febbre nei bacteri," 'Riforma medica,' 1893, etc.

[2] Golgi, in his remarkable studies on malaria, has interpreted the fever as the effect of the oxidation of the toxic products of the malarial amœbæ, products which are liberated at the moment of the segmentation precisely in coincidence with the beginning of the fever. According to us, it is very probable that the malarial hyperthermia is determined in part also by the destruction of a large number of the young amœbæ, which occurs exactly in correspondence with the commencement of the fever, as Golgi himself has demonstrated in his studies on phagocytosis in malarial infection ('Rif. medica,' 1888).

[3] Massart et Bordet, "Recherches sur l'irritabilité des leucocytes," 'Annales Pasteur,' 1891, fasc. 5.

action. Bianchi-Mariotti,[1] injecting filtered cultures of diverse bacteria, also observed a notable diminution of the red corpuscles.

It was already known that hæmorrhages frequently occur during the course of anthrax. The recent studies on anthrax toxins demonstrate that the hæmorrhages are due not to the large number of the bacteria, but to the toxins secreted by them; and not only in anthrax, but also in typhoid, in hæmorrhagic septicæmia, and in other infections the hæmorrhages are explained by the direct action of the bacterial toxins on the walls of the vessels. The pyrotoxin of Centanni, besides its febrigenous action, has the power of determining hæmorrhages, especially of the intestine.

Enlargement of the spleen is met with in almost all the acute infective diseases, but its origin is intimately connected with the action of the micro-organisms. In malaria the enlargement of the spleen is certainly connected with the phenomena of phagocytosis, the deposition in this organ of the pigmental residua of the dead parasites, or with the localisation in the same of the living malarial amœbæ, as Golgi studying the æstivo-autumnal fevers has demonstrated.

In other infections enlargement of the spleen owes its origin principally to the action of the bacterial toxins. In fact, Martinotti and Barbacci[2] and others observed the spleen enlarging under the action of sterilised toxins. This is understood when we remember that the same toxins stimulate the proliferation of the white corpuscles, and destroy a large number of the red corpuscles which are deposited in the spleen.

The bacterial toxins also have an extraordinary importance in determining *degenerative processes* in the parenchymatous organs.

In the liver Banti observed cirrhotic conditions following injections of pneumococci. Hanot has described with the name of infective patches islands of necrosis by coagulation

[1] Bianchi-Mariotti, "Wirkung der löslichen Producte der Mikroorganismen auf die Isotonie und dem Hemoglobingehalt des Blutes," 'Wiener med. Presse,' 1884, No. 36.

[2] Martinotti und Barbacci, "Acute Milzanschwellung bei Infectionen," 'Centrbl. f. allg. Pathol.,' 1890.

following the injection of diverse toxins, and especially of the tubercle toxins. In man tuberculosis generally determines a fatty liver; in animals, on the contrary, a cirrhosis. Charrin and Rüffer observed various degenerations of the liver by the action of the pyocyanic toxins.

The kidneys feel in an extraordinary way the action of the bacterial toxins. The staphylococci and the streptococci have great tendency to localise themselves in the kidneys, but other microbes perturb the function of these organs by means of their toxins alone. The diphtheria toxin determines a toxic nephritis; the cholera vibrios act in an analogous manner.

The pyocyanic toxins injected in small doses give rise to an interstitial nephritis; in some cases they have also produced amyloid degeneration. Amyloid degeneration of the kidney has also been observed in animals in cases of chronic tuberculosis, and following slow intoxications by the secretions of the staphylococcus.

We might multiply these examples,[1] but we shall confine ourselves to describing the action of the bacterial toxins on the nervous system.

We have already spoken in general of the action of the toxin of the *B. pyocyaneus*; we shall now add that Charrin, by means of the pyocyanic toxins, obtained in rabbits a disease similar to spastic spinal paralysis. Rodet and Courmont have found that certain products of the staphylococcus soluble in alcohol have a paralysing action on the heart and on the sensory nerves, while other products insoluble in alcohol exalt the reflexes. Guinard and Artaut, with diverse bacilli, including the pneumobacillus of cattle, have obtained a diminution of pressure, paralysis of the vaso-motors, and grave passive congestion of the intestine. Sirotinin obtained the same condition with the products of the typhoid bacillus, of *B. coli*, etc.

Through the action of the diphtheria toxins many authors have observed degenerations of the nerves. Ceni[2] has de-

[1] Compare: Claude, 'Lésions du foie et des reins déterminées par certaines toxines,' Paris, Carré, 1897.

[2] Ceni, "Effetti della tossina difterica sugli elementi del sistema nervoso." 'Boll. d. Soc. Medica,' Pavia, 1896.

scribed grave alterations of the central nerve elements; Roger,[1] Gilbert, and Lion, inoculating sterilised cultures of streptococcus, obtained sclerosis of the anterior horns of the medulla spinalis.

Finally we wish to record that Golgi,[2] by a series of remarkable works, has demonstrated the anatomical basis of rabies, illustrating the multiple alterations produced by the rabic virus in the cerebro-spinal axis.

Thus with this phase of studies, which certainly is not yet complete, the parasitic pathology integrates the cellular pathology. Only the union of these two doctrines has enabled us to discover the causes, to follow the genesis, the evolution, and the consequences of the infective diseases.

IMMUNITY.

When the organism is the victor in the struggle against the micro-organisms it obtains a precious advantage—*immunity* against the disease which it has passed through.

Acquired immunity is, then, the consequence of a mild infection undergone with favourable result. On the basis of such result experimenters have endeavoured, by reproducing at will such slight infections, to confer on animal organisms the power of resistance against the pathogenic germs by which they are threatened.

In this way originated the so-called preventive vaccinations that in a few years have had a very large practical application,—from chicken cholera to anthrax, from swine plague to diphtheria.

The preventive vaccinations are practised in diverse modes.

(A) With attenuated cultures obtained by various expedients. For example, heating the anthrax cultures at 42° C. from one to twelve days, a series of vaccines which are used in veterinary practice are obtained.

[1] Roger, "Atrophie musculaire progressive expérimentale," 'Annales Pasteur,' 1892.

[2] Golgi, "Sulle alterazioni del sistema nervoso nella rabbia:" a series of notes in 'Boll. della Soc. Med. di Pavia,' from 1887 to 1894.

Attenuated cultures are also obtained by treating the anthrax bacillus with bichromate of potash, the diphtheria bacilli with iodine terchloride. Other attenuations are obtained by drying; that of the rabic virus, for example. Others still are obtained by passing the culture through animals little sensitive, as the swine plague through rabbits. By old cultures immunity is conferred against virulent cultures of chicken cholera.

(B) Some vaccinations are obtained by inoculating small doses of *toxin;* Chauveau has done this for anthrax, Foà and Bonome for the proteus, Fränkel for diphtheria, etc.

(C) Other preventive vaccinations are obtained with the *proteins* of Buchner. To the proteins appertain tuberculin and mallein.

(D) Some immunisations are obtained by inoculating the so-called antagonistic bacteria. Thus, for example, Emmerich rendered rabbits almost insensitive to anthrax, inoculating them with slightly virulent streptococci.

(E) In fine, continuing the study of immunity, Richet, Hericourt, and, above all, Behring, Kitasato, and Ehrlich have discovered that in the blood of animals which have survived certain infections, agents were found antitoxic in respect to the germs and the toxins of the disease suffered. From this was derived a new curative and preventive method called *serum-therapy*, that with the treatment of diphtheria has triumphantly entered the clinic, and has been confirmed by all the observers of the world.

How is natural or acquired immunity in respect to infective diseases explained?

Contemporary pathology has not yet definitely answered this problem.

The observations obtained up to now have not permitted the formulisation of a well-founded doctrine *apropos*, inasmuch as they have given rise to more or less provisional hypotheses only.

These hypotheses are: the theory of phagocytosis, the humoral theory, the cellular-humoral theory.

The *theory of phagocytosis* put forward by Metschnikoff has already been mentioned in another part of this chapter.

According to Metschnikoff, the leucocytes attracted by the chemiotactic action of the toxic substances hasten to devour the microbes, thus tending to destroy them, and if they succeed in their office the animal is immune from the disease. When an infection is cured this occurs because the phagocytes succeed in destroying the parasites. The phagocytes thus gradually habituate themselves to the action of the parasites, and in case of a new invasion of the latter they are able rapidly to destroy them; that is, the animal has become immune. In his latest works Metschnikoff admits that the phagocytes also kill the bacteria in an indirect way, habituating themselves to elaborate in large quantity the alexins of Hankin, which are bactericidal and antitoxic substances.

The *humoral theory*, founded by Foder, Nuttall, etc., explains immunity by the presence of particular very unstable organic substances in the blood-serum, which are identical with Hankin's alexins. Kossel maintains that these substances are leuconucleins.

The *cellular-humoral theories* are those which now meet with greatest favour. It has already been known for some time that the organism can habituate itself to the toxins. Ehrlich has demonstrated that the blood-serum of animals habituated to toxins has an antitoxic action on the toxins themselves, so that if this serum be injected into a sensitive animal and the toxin is then inoculated, the animal survives. The neutralisation of the toxin also takes place *in vitro*.

An analogous but not identical fact is effected by certain bacterial toxins. Animals rendered artificially immune against an infection by means of repeated and increasing injections of the relative toxin furnish a blood-serum capable of preserving other animals. This is the famous discovery of Behring.

Behring has also demonstrated that the serum paralysed the morbigenous action of the bacteria and neutralised the toxic action of their toxins even when the disease had already commenced; the serum, therefore, is an antitoxin. This antitoxin being readily eliminated from the blood confers an immunity of short duration. On the other hand, the immu-

nity conferred by the injection of gradually increasing doses of toxin, or by the inoculation of vaccines, endures for a very long time, because it depends on the property acquired by the cells of the body owing to the stimulating action exercised by the toxin or by the vaccine.

To-day, however, the idea that the antitoxic serum neutralises the bacterial poisons without the participation of the cells is continually losing ground. Now-a-days the tendency is to believe that the antitoxin is a product of cellular activity, which in its turn has a particular action on the cells of the sensitive organism, and thus stimulates them to elaborate neutralising substances that, perhaps, are *leuconucleohistons*.

The future will decide this scientific controversy, but meanwhile serum-therapy remains as a precious gift which the pathology of the laboratory has given to the clinic.[1]

This is sufficient not only to justify the importance of experimental researches in medical studies, but also to accord them the right of supremacy.

On the question of immunity innumerable works have been published within the last few years. In order not to fill up many pages with bibliographical indications, we refer the reader to the *Ergebnisse* of Lubarsch and Ostertag, to the *Jahresberichte* of Baumgarten, and to special treatises on bacteriology.

[1] See Welch, "The Treatment of Diphtheria by Antitoxin," 'Transactions of the Association of American Physicians,' vol. x, 1895.

CHAPTER III.

COMPARISONS BETWEEN THE PRESENT DOCTRINE OF CELLULAR PROLIFERATION AND OTHER OLDER DOCTRINES.

MAURIZIO BUFALINI,[1] in his 'Foundations of Analytical Pathology,' justly observed that medicine was always, at least up till his time, governed by hypothesis, while the other natural sciences had much more quickly yielded to the severity of reason.

Physicians, instead of following the method of observation, attempted to penetrate the most recondite mysteries of the nature of living bodies, and they often fantastically imagined their causes, and arbitrarily delineated them in their effects. The wonderful discoveries of Malpighi and of Morgagni would easily have led anatomy, physiology, and medicine as a whole to the path of exact observations, were it not that the pernicious influence of the metaphysical philosophy attracted physicians for a long time to transcendental systems, apparently sublime, but in fact very far from the natural reality.

Thus, for example, we find that Haller[2] had placed the *fibre* as the foundation of the structure of the body. The elementary *fibre* in its turn was supposed to be composed of very thin fibrils visible only to the mental eye: "*invisibilis ea fibra, quam sola mentis acia adhingimus.*" According to Haller, the fibre was for the physiologists what the line was for the geometricians. This concept quickly spread, and the doctrine that the fibre served as the foundation of almost all the parts of the body, that the composition of the multiple tissues was reduced in the ultimate instance to the

[1] M. Bufalini, 'Fondamenti di Patologia analitica,' Milano, Truffi, 1833.

[2] Haller, 'Elementa physiologiæ corporis humani,' Losanna, 1757.

fibre, was established for a very long time, and applied even to the so-called cellular tissue. But after fifty years of uncontested dominion the doctrine of the fibre also, towards the end of the last century, began to find opponents in the so-called school of Natural Philosophers (Schelling).

The Natural Philosophers opposed to the system of fibres another speculative system, that of the *globule*, and we find that even Milne-Edwards,[1] at the beginning of this century, believed he had established that the fibre was composed of globules arranged in linear series. As Virchow[2] has well observed, the bad method which in the past century, and even in the beginning of the present century, was employed for observing with mediocre instruments in full light produced a notable dispersion of the luminous rays, and made the observer believe that he saw globules only. Such defect of observation confirmed the doctrine of the natural philosophers as to globules being the primary element of the organic tissues, and thus furnished the foundation for the doctrine of the blastema and of the molecular granules which organise themselves into cells according to the idea of Schleiden and Schwann, as we shall see further on. But at the beginning of this century the physical and chemical sciences, which made wonderful progress owing to the experimental method, were not slow in detecting the errors contained in the old hypotheses. They, besides, furnished new means of investigation and improved the old; it was owing to these means and their systematic application to the study of the organic tissues that, towards 1840, the cellular theory, from which Virchow derived the cellular pathology, was created.

The cell, already foreseen by Robert Hooke[3] in 1665, was really discovered by Marcello Malpighi in 1671, studying the vegetable tissues. Furthermore, Malpighi[4] then clearly dis-

[1] Milne-Edwards, 'Leçons sur la physiologie de l'homme et des animaux.'

[2] Virchow, 'La patologia cellulare fondata sulla dottrina fisiologica e patologica dei tessuti,' Milano, Vallardi, 1863.

[3] R. Hooke, 'Micrographia, or some physiological descriptions of minute bodies made by magnifying glass; with observations and inquiries thereupon,' London, 1665.

[4] M. Malpighi, 'Anatome plantarum,' 165; see also the 'Proceedings of the Royal Society,' London, 1871.

tinguished the cells in the vegetable tissues, which he called utricles or vesicles and tubes, that we now indicate by the name of vessels.

After Malpighi, Wolff observed the blood-islands in the vascular area of the embryo of the fowl, and there recognised distinct globules; he also studied the buds of plants, and maintained that these buds were constituted of a gelatinous substance, which afterwards collected into drops destined to be transformed into utricles, that is into cells.

We here have the first idea of the free formation of the elements.

Brisseau-Mirbel,[1] at the commencement of this century, had already adopted this theory, and he was the first who generalised the name of cell to the vegetable elements studied; he recognised, besides, that the vegetable vessels and fibres are a derivation of the cells.

Treviranus[2] also had a precise conception of the cells; he maintained, however, like Mirbel, that the cells were excavated in a homogeneous fundamental substance. The first who had the idea of the anatomical individuality of the cellular elements was Moldenhauer,[3] who by means of macerations had succeeded in isolating the vegetable cells, and in demonstrating that each cell had a wall proper.

Some years later Turpin's work[4] appeared, to which Sangalli[5] justly accords notable importance.

It is sufficient to refer to the title of Turpin's work to show how precise his ideas were on the cells and on cell proliferation.

His memoir, in fact, bears the long title 'Organographie microscopique élémentaire et comparée des végétaux: observations sur l'origine et la formation primitive du tissu

[1] Brisseau-Mirbel, 'Exposition de la théorie de l'organisation végétale,' Paris, 1809.

[2] Treviranus, "Vom Inwendigen," 'Bau der Gewächse,' 1806.

[3] Moldenhauer, 'Beiträge zur Anatomie der Pflanzen,' 1812.

[4] Turpin, 'Organographie microscopique élémentaire des végétaux,' Paris, 1826.

[5] Sangalli, 'La scienza e la pratica dell' Anatomia patologica,' Milano, libro iv, pp. 22 and following.

See also Robin, 'Anatomie et Physiologie cellulaires,' Paris, Baillière, 1873, pp. 558 and following.

cellulaire, sur chacune des vésicules composantes de ce tissu, considérées comme autant d'individualités distinctes ayant leur centre vital particulier de végétation et de propagation, et destinée à former par agglomération l'individualité composée de tous les végétaux dont l'organisation de la masse composte plus d'une vésicule.'

Turpin was, perhaps, the first who established that the organisation of a living being, and that of its organs in particular, could be understood only by following the gradual development of this being from the first moment of its formation to its death. After having stated that it was impossible to have exact ideas of the nature and the analogies of organised beings until complex and adult beings were studied, he admitted that every organism, including man, is always a species of composition of entities more simple than it.

We therefore already find in Turpin the federal concept of Virchow, the doctrine of the elementary organisms which was subsequently treated physiologically by Brücke. With regard to the genesis of the cells, however, Turpin's ideas were not very clear; he, indeed, wrote, "Every plant, like every being whatever, begins as a globule; this globule, the propagator of its nature, becomes hollow internally, and is transformed into a vesicle; from the internal walls of this vesicle arises by extension a new generation of globules equally propagators. These growing fill all the capacity of the mother vesicle, which cannot contain any more of them, so that it bursts, and a numerous generation of individuals issues forth, which form a mass more or less united together, and they in their turn continue to generate new individuals, to multiply in number and increase the extension of the mass." At this point it is necessary also to record even briefly the studies of an illustrious Italian, Mauro Rusconi,[1] who, though he did not know the cellular theory, has described in a very exact manner the segmentation of the ovum of the frog and of the salamander; he was thus the first who has seen in the most certain way the proliferation of the cells.

[1] Mauro Rusconi, 'Sur le développement de la grenouille, etc.,' Milano, Giusti, 1826.

Meyen,[1] in a treatise on botany which appeared in 1830, expresses, in the constitution of organised beings, ideas very similar to those of Turpin. The vegetable cells, he says, are sometimes isolated, and then each one of them constitutes an individual, as is the case of the most simple algæ and fungi; other times, on the contrary, the cells are united into a more or less voluminous mass to constitute a vegetable of higher organisation. But in this case each cell feeds by itself, is formed by itself, and transforms the brute matter into very different substances and organs.

Even the constitution of the cell was partly known by the relatively old observers. Fontana [2] had exactly recognised the nucleus in the epithelial cells of the eel; furthermore, he had also seen the nucleus (1781), inasmuch as he describes the nucleus as a round oviform body, provided with a little body in the centre. Cavolini [3] also, in 1787, recognised the nucleus in the ova of fishes. The botanist Robert Brown,[4] in 1833, recognised that this nucleus is a normal element of the cell.

In 1837 Valentin [5] described the corpuscle contained in the cells of the conjunctiva, and gave it, for the first time, the name of nucleus; in its turn he also described a round corpuscle within the nucleus, the nucleolus, "forming a kind of second nucleus within the nucleus."

We finally arrive at the generalisation of the cellular theory initiated by Schleiden,[6] and his theory on the free formation of the elements. Schleiden, in a celebrated work published in Müller's Archives, pushing forward the researches of his predecessors, and principally those of Brown, demonstrated, above all, the existence of the nucleus in a large

[1] Meyen, 'Anat.-phys. Untersuchungen über d. Inhalt. d. Pflanzenzellen,' Berlin, 1828.

[2] Fontana, 'Traité sur le venin de la vipère, avec des observations sur la structure primitive du corps animal,' Firenze, 1781.

[3] Cavolini, 'Memoria sulla generazione dei pesci,' Napoli, 1787.

[4] R. Brown, 'Observations on the Organs and Mode of Fecundation in Orchids,' Linnean Society, London, 1831.

[5] Valentin, 'Repertorium für Anatomie und Physiologie,' vol. i, Bern, 1837.

[6] Schleiden, "Beiträge für Phytogenesis," 'Muller's Archiv für Anat. und Physiol.,' 1838.

number of vegetable elements, and he especially verified its constant presence in the young cells. This discovery made him think that the nucleus must be in intimate relation with the mysterious formation of the cells; it must, consequently, have an essential importance during the whole cellular life. He positively maintained that the nucleus was the generator of the cell, and he therefore gave it the name of cytoblast.

To explain the origin of cells, Schleiden took up the old theory of the natural philosophers on the elementary globules and granules; that is, he admitted that in the centre of an amorphous fundamental substance a granule is first formed,—that is to say, a nucleolus; other granules, collecting round the first, give rise to the formation of a nucleus or cytoblast. On the surface of the cytoblast the cellular membrane, which covers the former like a watch-glass, is subsequently formed. The membrane then increases, separates from the nucleus, so that a vesicular cavity forms between them, into which the fundamental substance gradually penetrates, filtering, so to speak, through the membrane.

The accurate anatomical observations of Schleiden caused the cellular theory to be accepted by botanists; almost at the same time the cultivators of general anatomy were building up the cellular theory of the animal organisms.

Some years before the remarkable work of Schleiden was published, Purkinje[1] and Valentin had compared certain animal tissues to vegetable tissues, and recognised the cellular structure of the epithelia; John Müller,[2] studying the comparative anatomy of myxons, recognised the cellular structure of the chorda dorsalis and of cartilage; Henle,[3] studying the structure of the intestine, verified the cellular constitution of the glands.

But Schwann was the first who established a cellular theory truly applicable to all the animal tissues. Schwann, subsequent to a learned conversation which he had had with

[1] Purkinje, 'Bericht über die Versammlung deutscher Naturforscher und Aerzte in Prag, in September, 1837.'

[2] J. Müller, "Vergleichende Anatomie der Myxinoiden," 'Müller's Arch.,' 1837.

[3] Henle, 'Symbolæ ad Anatomiam villorum intestinalium,' 1837.

Schleiden, accepted the latter's doctrine on the formation of cells, and on the cellular structure of vegetables.

Schwann quickly understood—as he himself tells us in his first celebrated work—all the importance that a methodical comparison between the animal and the vegetable tissues must have. With admirable ardour, Schwann undertook an innumerable series of comparative researches, which he published in 1839, expounding them in a truly able form.[1]

Two circumstances contributed essentially to the great success of Schwann's work. First of all, he verified the constancy of the nucleus in the greater number of the animal cells, and concluded that the nucleus is the least variable and the most characteristic element of the cell. In the second place, Schwann, with truly happy intuition, found the method most adapted for solving the problem of the constitution of the tissues. As Turpin and Schleiden had done for the vegetables, he recognised that it was necessary especially to study the development of the diverse tissues; he was thus able to verify that man at the commencement of his development consists of a mass of entirely similar cells. He then followed in large lines the metamorphoses which the embryonic cells undergo by being transformed into the tissues of adult animals, and was thus able to show that while some cells present a spherical form, others become cylindrical, others stellate, others are transformed into long fibres. He also recognised that some elements acquire very thickened walls,—as in cartilages, bones, teeth; in fine, he explained that certain very differentiated tissues (nerve-fibres) are formed by a fusion of cells precisely as the vegetable vessels are.

As regards the genesis and the proliferation of cells, Schwann accepted the ideas of Schleiden in full,—that is, he admitted the free formation of the elements. He thought that between the pre-existing cells, and even within them, existed a special amorphous matter, the *cytoblastema,* which by its chemical composition was capable in a more or less high degree of giving rise to the formation of cells.

[1] Schwann, 'Mikroscopische Untersuchungen über die Uebereinstimmung in der Structur und dem Wachstum der Thiere und Pflanzen.' 1839.

He accepted, to a certain extent, the doctrine of Raspail,[1] who maintained that the formation of cells is nothing more than a vesicular crystallisation. Schwann, like Raspail, wrote, "*Die Zellenbildung stellt für die organische Natur dasjenige dar, was für die anorganische die Krystallisation ist.*"

First of all, said Schwann, in the cytoblastema is formed a granule, the nucleolus, which acts attracting the particles that are around it, and it thus clothes itself with a new stratum of substance; in this way the nucleus is formed by the nucleolus.

The process of attraction is repeated around the nucleus, and thus a second stratum is formed, which at first is not very distinct from the surrounding mass. Then this stratum thickens and forms the cellular substance and membrane. At first the latter is very close to the nucleus, and the cell appears very small; then the membrane swells, and the cell acquires its specific contents.

In other cases the formation of the cell takes place in a slightly different mode, so that elements are produced with two nucleoli or with two nuclei.

Schwann's theory on the free formation of the elements agrees in a positive way with the doctrines of the old surgeons on the genesis of pathological neoplasias. Indeed, long before the appearance of the cellular theory the celebrated English surgeon, John Hunter,[2] had expressed the opinion that the point of departure for every neoplasia is always an effusion of plastic lymph. The plasticity, a property inherent in the effused matter, renders this matter capable of forming spontaneously all kinds of tissue.

Hunter compared the development of a pathological neoplasia with the evolution of the chick in the ovum, and, not knowing the cellular theory, he maintained that the first elements developed were those of the blood-vessels, from which new quantities of plastic lymph were subsequently derived. Thus Hunter maintained to a certain extent that

[1] Raspail, 'Nouveau système de chimie organique,' Paris, 1838, vol. ii, p. 403.

[2] J. Hunter, 'A Treatise on the Blood, Inflammation, and Gunshot Wounds,' London, 1794.

neoplasms were formations extraneous to the organism, and, so to speak, parasites.

The plastic lymph of Hunter survived, however, under the form of the cytoblastema of Schwann, even after the discovery of the cellular doctrine.

Somewhat distinct from this doctrine, but still closely connected with it, was the theory developed by Arnold [1] of the elementary granules and molecules; later Arnold united this doctrine with the free formation of the elements, maintaining that a cell was formed by the disposition of the granules into a spherical form, subsequently reduced into a membrane containing other globules, which gave rise to the nucleus and the cellular body.

The Vienna school, with the celebrated Rokitansky [2] at its head, also adopted the doctrine of free formation to explain the pathological neoformations.

In typhoid, for example, he maintained that an albuminous exudate filled the interstices of the tissues and organs, and that from this exudate exactly their swelling and medullary aspect were derived; this exudate then was capable of taking form and giving rise to numerous cells by a kind of equivocal generation.

Luschka [3] explained in the same way the genesis of pus cells. According to this and other authors, in the lymph of pus simple granules first appeared, capable of aggregating and forming larger bodies, which, by the appearance of a nucleus in their centre, were gradually transformed into distinct cells.

Bruch [4] also admitted that the nuclei were first formed by the fusion of the elementary granules. These nuclei were then surrounded by a badly defined precipitate, round which a membrane developed. The nuclei, however, once formed, subsequently developed according to the doctrine of proliferation, of which we shall subsequently speak.

[1] Arnold, 'Lehrbuch der Physiologie des Menschen,' Theil 2, Zürich, 1842.

[2] Rokitansky, 'Handbuch der pathologischen Anatomie,' Wien, 1842–55.

[3] Luschka, 'Entwickelungsgeschichte der Formbestandtheile des Eiters und der Granulationen,' Freiburg, 1845.

[4] Bruch, 'Die Diagnose der bösartigen Geschwülste,' etc., Mainz, 1847. Compare also Günther, 'Lehrbuch der allgemeinen Physiologie,' 1845.

Precisely in this period, towards 1850, the idea, already expressed by many authors, which we shall presently allude to, according to which the cells are derived from the proliferation of pre-existing cells, began to gain ground. In 1841 Remak [1] considered division as the only mode of multiplication of the cells, and he maintained that free formation had no foundation in fact.

Unger [2] also, like Ugo Mohl,[3] had already observed the division of the cells, and he energetically combated the doctrine of free formation, defended in botany by Schleiden.

Kölliker,[4] in 1844, studying the development of the Cephalopods, recognised that even in the embryo all the cells are derived by continuous division from the segmentation spheres; on the basis of these observations he completely denied free formation even for the adult tissues, admitting that all the cells were direct descendants of the segmentation spheres.

In 1846 Nägeli [5] formulated the general law for the vegetable cell. According to this law the new vegetable cells are always exclusively formed at the expense of the pre-existing cells, and precisely the mother cells give rise to two or more daughter cells by a process of division which was observed for the first time by Ugo Mohl.

Reichert [6] and Vogt [7] acquired an equal conviction by the study of the animal tissues, while observations accumulated on the verified facts of nuclear division, which are those described by Valentin and Henle.[8]

[1] Remak, "Theilung rother Blutzellen beim Embryo," 'Med. Vereins-zeitung,' 1841.

[2] Unger, 'Grundzüge der Anatomie und Physiologie der Pflanzen,' Wien, 1846.

[3] Ugo Mohl, 'Ueber die Vermehrung der Pflauzenzellen durch Theilung,' Dissert., Tübingen, 1835.

[4] Kölliker, 'Entwicklungsg. der Cephalopoden,' Zurich, 1844, pp. 111—160.

[5] Nägeli, "Zellkern., Zellbildung und Zellenwachsthum bei den Planzen;" in Schleiden und Nägeli, 'Zeitschrift für wissenschaftl. Botanik.'

[6] Reichert, "Der Furchungsprocess u. die sogenannte Zellenbildung um Inhaltsportionem," 'Müller's Archiv,' 1846.

[7] Vogt, 'Embriologie des Salmones,' Neuchatel, 1842.

[8] Henle, 'Archiv für Anatomie und Physiologie,' 1843.

After the accumulation of numerous observations, Remak [1] published his remarkable studies on segmentation, on the development of the embryo, and on the genesis of the animal cells. He verified in a definite way that a free formation of elements does not take place in any part of the embryo; that all the cells of the blastodermic layers are derived from the segmentation of the vitellus, and that it is by a continuous division of these cells that the elements constituting the diverse organs and tissues are produced and multiplied.

This generalisation of the doctrine of the proliferation of cells in the normal tissues was readily extended to the pathological tissues, inasmuch as isolated observations of cellular divisions met with in tumours were not wanting.

Among others I am pleased to mention those of Günsburg [2] and Breuer,[3] who, in 1843 and 1848 respectively, described in a precise way the divisions of the nuclei in pathological tissues, and interpreted them as signs of cellular proliferation.

Moreover, J. T. Goodsir,[4] of Edinburgh, not only applied to pathology the doctrine of cellular proliferation, but he also traced in its broad lines, though in a somewhat indeterminate manner, the entire cellular pathology.

The ground was therefore well prepared for the reception of the new doctrine; the numerous researches of embryologists had given a very solid and generally recognised foundation to the doctrine of proliferation in the normal tissues; the various observations of diverse pathologists had shown that the same law must also hold good in the pathological field.

Virchow, after having verified many fundamental facts on the pathological proliferation of cells, after having described this process in such a very minute way as to indicate unconsciously the chief facts of the later karyokinetic doctrine,

1 Remak, "Ueber extracellulare Entstehung thierischer Zellen und über Vermehrung derselben durch Theilung," 'Müller's Arch.,' 1852.

2 Günsburg et Breuer, 'Meletemata circa evolutionem ac formas cicatricum,' Vratislaviæ, 1843.

3 Breuer, 'Die pathologische Gewebelehre,' Leipzig, 1848.

4 J. T. Goodsir, 'Anatomical and Pathological Observations,' Edinburgh, 1845.

especially in the study of the division of cancer cells,[1] published in 1858 his memorable book on 'Cellular Pathology;'[2] and, by formulating the axiom *Omnis cellula e cellulâ*, generalised the law of Kölliker and Remak also for the pathological tissues.

Objections to the Doctrine of Proliferation.

Although Virchow's work had an extraordinary success, and his doctrine was received, defended, and promulgated, sometimes even amplified by innumerable followers, still there were not wanting even later on, especially among pathologists, scientists of great authority who maintained the doctrine of free formation, either in an exclusive way or in connection with the doctrine of proliferation.

Broca,[3] for example, professed tenaciously the theory of the blastema. For him this substance emanated directly from the blood, from which it separated, traversing by exudation the parietes of the capillaries. The blastema, therefore, begins by being absolutely liquid, but later on it passes into the solid state. This change of state may be effected in two modes: sometimes it is the result of a coagulation pure and simple, which precedes the organisation proper; at others, on the contrary, the solidification is effected by the act of organisation. During the variable period that precedes this solidification the blastema filters by imbibition into the intervascular spaces. If the place where the exudate occurs be very near the free surface of a membrane, then the blastema exudes through the membrane and expands on its surface; if, on the other hand, it finds itself in contact with a tissue deprived of vessels, it saturates the whole substance of this tissue. This is the mechanism by which the non-vascular organs receive the normal blastema (plasma) necessary for their nutrition and

[1] R. Virchow, "Ueber die Theilung der Zellenkerne," 'Virchow's Arch.,' 1857, Bd. ii, S. 89. Cf. also Flemming, "Ueber das Verhalten des Kerns bei der Zelltheilung," etc., 'Virch. Arch.,' vol. lxxvii.

[2] R. Virchow, 'Cellulare Pathologie,' Berlin, 1856.

[3] Broca, 'Traité des tumeurs,' vol. i. p. 84.

growth: the pathological elements which sometimes form in such organs prove that the abnormal blastemæ penetrate into the tissues in the same way.

Robin,[1] in 1864, and even up to 1868 and 1873, also maintained the theory of the blastema. His theory is a little more complex than that of his predecessors, but it agrees in the main with the conception of the free formation. Robin, in fact, in 1864 admitted that in the depth of a liquid, or in the midst of the anatomical elements, certain immediate principles of the plasma, formative humour, or blastema, unite almost suddenly, molecule to molecule, so as to form a solid or semi-solid substance, amorphous or figured.

Without being derived directly from any of the surrounding elements, the new individuals arise in the tissues by new generation (*par génération nouvelle*) at the expense of the principles furnished by the tissues themselves, principles which associate according to the determinate laws of molecular attraction. To this mode of origin of the organised substance Robin gave the name of *genesis*.

In other writings Robin[2] expresses himself a little differently; he always admits the free formation of the elements, but he maintains that this occurs only in the midst of the pre-existing anatomical elements. For him, if the genesis of the anatomical elements be a spontaneous generation, inasmuch as it consists in the appearance of organised particles where they had not previously existed, it is necessary to admit also that, owing to the conditions in which such appearance takes place, such genesis is altogether distinct from heterogeny,—that is, from the production of entities in an unorganised cosmic medium. In molecular renovation the act of assimilation consists in the formation of immediate principles similar to those that constitute the anatomical element; such principles are, therefore, a little different from those of the blood-plasma. When this assimilative formation exceeds the disassimilation there is an increase of mass of the element. But this formation of principles very quickly extends

[1] Robin, "Mémoire sur les divers modes de naissance de la substance organisée," etc., 'Journal de l'Anatomie,' 1864, p. 43.

[2] Robin, art. "Blastema," 'Dictionnaire encyclopédique des sciences médicales,' Paris, 1868.

beyond the element, inasmuch as the excess of the formative principles cannot accumulate within the element after the latter has reached a certain degree of development. These principles in excess collect between the elements themselves, and considered in their *ensemble* and in their association in a liquid or semi-liquid whole they receive the name of *blastema*. As they gradually form, such principles must organise themselves, combining molecularly into a substance which may be amorphous or shaped into nuclei, cells, etc.[1]

Besides these affirmations of a rather *doctrinaire* and speculative nature, descriptions of discoveries relative to free formations have not been wanting even in comparatively recent times.

Thus Oehl[2] admitted the free formation of leucocytes in the course of his interesting studies on inflammation, of whose value, in respect to the doctrine now generally accepted, we have spoken in another chapter.

Onimus,[3] in 1867, thought he saw the free formation of leucocytes in particular experimental conditions; and Montgomery has also described a free formation of cells within the animal body.[4] Nevertheless the experiments of Onimus and the observations of Montgomery have been severely criticised, and their results have been considered incredible even by authors who did not particularly support an opposite doctrine. The researches of Ganin[5] alone on the development of the ovum of the Platygaster (a species of Hymenoptera), published in Kölliker's 'Archives,' seemed to prove in certain cases the reality of a free cellular formation.

But Kölliker[6] himself, in the last edition of his treatise, observed that after his own studies and those of Remak, and above all after the generalisation of the principle *Omnis*

[1] Robin, 'Anatomie et physiologie cellulaires,' Paris, Baillière, 1873.

[2] Oehl, "Fisiologia del processo infiammatorio," estr. dalla 'Gazzetta medica italiana,' Lomb., t. iv, 1865.

[3] Onimus, "Expériences sur le génèse des leucocites," 'Journal de l'Anat. et de la Phys.,' 1867.

[4] Montgomery, 'On the Formation of the So-called Cell in Animal Bodies,' London, 1867.

[5] M. Ganin, "Beiträge zur Erkenntniss der Entwickelungsgeschichte der Insecten," 'Zeit. für wiss. Zoologie,' 1869, xix.

[6] Kölliker, 'Handbuch der Gewebelehre,' Leipzig, 1889.

cellula e cellulâ of Virchow, the majority of observers had abandoned the doctrine of free formation. It is very true, says Kölliker, "that from time to time some author refers to data which appear to speak in favour of that doctrine, but positive proofs demonstrating the existence of a true free formation have never been given by anyone."

Even Arnold's [1] data, according to which, following a loss of substance, a neoformation of epithelial cells is effected by a simple segmentation of the protoplasm without the participation of the nucleus, which appears later on in the protoplasmic particle transformed into a cell, have by no means been confirmed; on the contrary, they are in direct contradiction to the very recent researches of Balbiani [2] and others on merotomy.

In fact, nowadays by the numerous researches of Grüber, Nussbaum, Hoffer, Verworn, etc., it has been demonstrated that if a cell or a unicellular infusorium be divided into two parts, one of which contains the nucleus, the other being deprived of it, only the part containing the nucleus is capable of growing and multiplying. The other part may live for a variable time (up to fourteen days), but it cannot complete itself, nor grow, nor multiply; it thus ends by succumbing. The nucleus, consequently, is an indispensable part of the cell.

Finally, it is necessary particularly to mention the eclectic doctrine professed by Sangalli.[3] This author, though an extreme follower of the theory of free formation sustained and defended by him in various writings, in which he has described the organisation of the fibrin into connective-tissue cells, and even into elements of sarcoma and of cancer, or into pus cells, admits, however, in diverse works, including his treatise, the possibility of pathological proliferation.

Indeed, in his 'Clinical and Anatomical History of

[1] J. Arnold, 'Virchow's Arch.,' Bd. xlvi, p. 168, 1869.

[2] Balbiani, "Recherches expérimentales sur la mérotomie des Infusoires ciliés," 'Recueil Zool. Suisse,' 1889.

[3] Sangalli, "Esame di due casi di cancro ad illustrazione della storia dello sviluppo dei tessuti," 'Giorn. del R. Ist. Lomb.,' 1854, p. 207. Idem, "Coagulazione del sangue durante la vita, varie organizzazioni successive di esso, varie successioni di organizzazione dei tessuti morbosi," ibid., 'Mémoire,' vol. xii, p. 181, 1871.

Tumours' he[1] states that he had observed the development of the morbid epithelial elements of the stratum Malpighii of the epidermis, and in a lively lecture on the cellular pathology, describing the history of and criticising the same, he admitted, with Rokitansky, the existence of mother-cells capable of generating other cells. In this connection he referred to his own observations on the dendritic vegetations in cancers.[2] Also in his great treatise on pathological anatomy,[3] speaking in general of the origin of cells, he admits in part a pathological proliferation. In fact, he writes, "It is here opportune to explain the signification of such vegetation or multiplication of the elements, since I give them a value a little different from that of true proliferation. Since we do not always see a production of cells within other cells; since the derivation of one cell from another is not always clear, as occurs in the spores of favus and of thrush; since we appear rather to see a development of heaps of cells and of nuclei piled on one another without visible dependence: so for the word proliferation I prefer those of vegetation and multiplication. In this sense I also accept the terms cellulation and nucleation adopted by the followers of proliferation, because they do not express the idea of a true proliferation. I think likewise that the conception of the division of the nuclei has a wider and a more acceptable meaning than that of proliferation."

"In connection with these ideas I admit that small round elements are found within an inflamed tissue; and I maintain that they express a proliferation in a wider sense, truly speaking, than that which is generally accepted by the followers of this theory. The field of the endogenous production is larger than that of a cell, but the connective substances and the intercellular protean humour, as well as the protoplasm of the cells, are very active and capable of developing into small elements." As is seen, Sangalli here admits in connection with the theory of proliferation also the theory of free formation, with the restrictions already made by Robin. It is to be remarked, however, that he very

[1] Sangalli, 'Storia clinica ed anat. dei tumori,' vol. i, p. 48, 1861.

[2] Sangalli, "La patologia cellulare riscontra con i fatti anatomici e clinici," 'Lo sperimentale,' vol. x, 1862.

[3] Sangalli, 'La scienza e la pratica dell' Anatomia patologica,' lib. iv.

willingly records in his treatise his own observations and those of others on the pathological proliferation of cells, and that in Book V of the same work he admits even more precisely pathological proliferation and its importance.[1]

Parasitism of Tumours—Heterology—Specific Cells.

With the theory of free formation of the elements in pathological tissues we believe it necessary to associate the doctrine of the heterology of tumours, and that of the so-called specific cells.

We have mentioned already that John Hunter thought that the origin of every neoplasm was to be sought in an effusion of *plastic lymph*, capable of organising itself. This plastic lymph is evidently the same as the *cytoblastema* of Schwann.

In this plastic lymph an independent circulatory system was supposed to be afterwards developed. J. Hunter[2] compared pathological neoplasms with the development of the chick in the egg, and attempted to prove that, since the *punctum saliens* in the egg shows the blood-vessels as the first phenomenon of life, so in a neoplasm the blood-vessels are the first which appear and give a special vital character to the pathological production in formation. These considerations led Hunter to regard a neoplasm as an extraneous production added to the organism,—that is to say, as a kind of *parasite*. He, consequently, revived the old idea of Harvey.[3] This doctrine agreed well with the doctrinaire element of Schönlein's school, which considered disease as an entity *per se*, distinct from the organism. In accord with Hunter's doctrine, Bichat in France, and Rust and Kluge[4] in Germany, described parasitic neoplasms as furnished with an independent vascular system, which, without having origin or roots in the pre-existing vessels, was formed

1 Sangalli, 'La scienza e la prat. dell' Anat. patol.,' lib. v, pp. 13, 14.

2 Hunter, l. c.

3 Harvey, 'Exercitationes de generatione animalium,' Amsterd., 1851.

4 Kluge, quoted by Virchow, 'Die krankh. Geschw.,' Berlin, Hirschwald, 1863, vol. i, p. 22.

by itself alone, as in the chick. Cruveilhier,[1] much more positive, did not accept the parasitism of tumours; he admitted, however, that cancers especially were something heterologous in respect to the organism.

This idea by some was carried so far that they maintained that tumours were entozoa produced by spontaneous generation within the organism. And the observations made at that time on hydatid cysts furnished to a certain extent an objective basis for the above-mentioned doctrine. The acephalocysts indicated the passage of the tumours due to cestoids towards cysts and tumours of an indeterminate nature, and they helped thus to confirm the parasitic interpretation of neoplasms. Be this as it may, the authors agreed in believing that a large number of tumours did not correspond to the parts of the body, and that at most they had only a distant analogy to the normal tissues.

Although Ehrenberg[2] had tried to establish the identity between the elements of tumours and those of the normal tissues, still the doctrine of parasitism prevailed for a long time, especially in France owing to the work of Dupuytren[3] and of Laennec.[4]

Lobstein[5] endeavoured to establish a division between the tumours which correspond to the normal tissues, and those which appear foreign to the organism. He thus divided tumours into *homeoplastic* and *heteroplastic*. Although, in Germany, Fleischmann[6] had recognised that tumours are only copies of the organic parts of the body in which they exist and develop, although J. Müller[7] described very well for his time the structure of tumours, nevertheless J. K. Meckel[8] and Hensinger[9] maintained Lobstein's ideas, and

[1] Cruveilhier, 'Essai sur l'anatomie pathologique,' 1816.

[2] Ehrenberg, cited by Virchow, 'Pathologie des Tumeurs,' vol. i, Paris, 1867.

[3] Dupuytren, 'Bulletin de l'École de Médecine de Paris,' 1805.

[4] Laennec, 'Traité de l'auscultation médiate,' 1837.

[5] Lobstein, 'Traité d'anatomie pathologique,' Paris, 1829.

Fleischmann, 'Leichenöffnungen,' Erlangen, 1815.

[7] J. Müller, 'Die krankhaften Geschwülste,' Berlin, 1838.

[8] F. Meckel, 'Handbuch der path. Anat.,' Leipzig, 1818, B. ii, Abth. 2.

[9] Hensinger, 'System der Histologie,' Eisenach, 1822.

made them triumph for the moment over the tentatives of Fleischmann and Müller.

Virchow alone, later on, taking up the ideas of these authors, confirmed their exactness, while accepting the names of homeoplastic and heteroplastic tumours, but in a very different sense from that primary, that is in the sense only of metaplasia of the tissues.

The heterologous tumours were considered as the expression of a profound alteration of the organism,—that is to say, of a particular dyscrasia capable of giving rise to a morbid condition diametrically opposite to physiological life. It thus happened that some endeavoured even to isolate chemically some specific matter, but as they failed to isolate any particular body, but only albumin, it came to pass that Rokitansky [1] thought that this albumin was the cacoplastic substance, a species of diseased albumin. In relation to this doctrine also is the conception expressed by Sangalli, who maintained that morbid-tissue tumours were caused by a process of perverted local nutrition. This conception, expressed repeatedly by Sangalli in his earliest work on tumours,[2] in his treatise, and in diverse memoirs,[3] agrees with his ideas on free formation, and with his ideas on the reproduction of tumours in distant parts. He maintains, in fact, that the infection of the organism does not in reality take place by the penetration of the cells and the nuclei of the primary tumour into the mass of the tissues; but by the humour which is generated in the primary morbid tissue when it has been absorbed by the lymphatics and by the small peripheral veins. For him a particular disposition to the repetition of the morbid tissue is generated in the patient.

The discovery of the sought-for specific substance being wanting, authors applied themselves—always on the basis of the above-mentioned dominating doctrines—to the quest for the *specific elements*. Thus it happened that many authors made themselves see special cells in tumours which gave

[1] Rokitansky, 'Handbuch der allgemeinen patholog. Anatomie,' Wien, 1846, p. 530.

[2] Sangalli, 'Storia clinica ed anatomica dei tumori,' 1861.

[3] Sangalli, "Osservazioni e raffronti sopra argomenti generali circa la patologia dei tumori non infiammatorî," 'Rendiconti dell' Ist. Lomb.,' 1886.

them a characteristic note. The supposed discovery of specific cells took place in Germany through the work of Gluge,[1] who described caudate corpuscles—evidently young connective-tissue cells—as being frequent in cancerous and sarcomatous tumours. This theory, accepted and developed in Germany by Hannover[2] and Bruch,[3] and also by Meckel,[4] was immediately combated by Müller[5] and Julius Vogel,[6] but it was warmly received in France, where it became diffused and almost universally accepted owing to the work of Lebert.[7]

The success of this theory is understood when one thinks that it was founded on the dominating doctrine of free formation and of the parasitism of tumours, through which it was permitted to consider a neoplasm as something distinct from the body, something that might be interpreted as a new creation.

Virchow,[8] in Germany, combated with particular energy the specific cells of cancer, departing from the doctrine of pathological proliferation. In Italy, with like fortune, Sangalli[9] combated this theory from his point of view. He, in 1852, at a meeting of the Lombard Institute immediately recognised that the doctrine of specific cells, though very plausible, did not resist the test of facts. And thirty-two years afterwards, in another lecture at the same Institute, recording with satisfaction his old observations, he brought forward fresh evidence to prove that pathognomonic anatomical characters do not exist in cancer or in other tumours.

The Plastidular Theory.

The old theories on the free formation of the elements, and those on the *globules* of Milne-Edwards, are connected

[1] Gluge, 'Pathologische Histologie,' Jena, 1850.

[2] Hannover, 'Müller's Arch.,' 1844. See also 'Das Epitelioma,' Leipzig, 1852.

[3] Bruch, 'Die Diagnose der bösartigen Geschwülste,' Mainz, 1847.

[4] Meckel, 'De pseudoplasmatibus in genere et de carcinomate in specie,' Halle, 47.

[5] J. Müller, 'Die krankhaften Geschwülste,' Berlin, 1838.

[6] J. Vogel, 'Pathol. Anatomie des menschlichen Körpens,' Leipzig, 1845.

[7] Lebert, 'Physiologie pathologique,' Paris, 1845.

[8] R. Virchow, 'Virchow's Arch.,' vols. i and vi, 1847, 1854.

[9] Sangalli, Istit. Lomb., 1852 and 1884.

without doubt with the more recent doctrines formulated by Béchamp, Maggi, Estor, and Altmann.

Béchamp[1] has supported the doctrine of the *microzymes*, that is of the elementary granules, and has associated these granules with the spontaneous generation of the cells and of the micro-organisms.

Maggi[2] has founded the doctrine of the *plastidules*. According to Maggi, the bacteria represent the plastidules or the independently living elementary granules; the cells constituting the organisms of the Metazoa are not elementary organisms, but colonies of plastidules associated together according to determinate laws.

Estor[3] also maintains that the cells are constituted of elementary granules, and Béchamp likewise admits that these granules again become free by a process of regression of the cell, and give rise anew to the formation of bacteria.

Maggi, in his more recent writings, has given a more philosophical and more complex explanation of the plastidular theory, interpreting the constitutive plastidules of the cell as *virtual* micro-organisms, and explaining the pathological processes of the elements as a disturbance of the laws of colonisation of the plastidules or a primary alteration of the fundamental activities of the plastidules.

Very analogous to Maggi's doctrine is that recently expounded by Richard Altmann,[4] and supported by elegant and minute observations.

According to Altmann, the individuality of the cell and its great significance for the interpretation of life cannot be denied. But the cell does not represent the most simple morphological unity of living matter; this must be sought in the elementary granules or *bioblasts*, very demonstrable in every cell by particular methods, which now take the

[1] Béchamp, 'Comptes rendus,' *passim* from 1860 onwards. Id., 'Les microzymes dans leurs rapports avec l'hétérogénie, histogénie, la physiologie, et la pathologie,' Paris, Baillière, 1883.

[2] Maggi, "Intorno ai protisti," etc., 'Boll. Scient.,' 1881, p. 113. Id., 'Protistologia,' Milano, Hœpli, 1893.

[3] Estor, 'De la constitution élémentaire des tissus,' Montpellier, 1882.

[4] Altmann, 'Die Elementarorganismen und ihre Beziehungen zu den Zellen,' Leipzig, Veit, 1894.

name of Altmann. The cells are for Altmann colonies of bioblasts; they are no longer formed spontaneously by the direct association of the granules, but they had such origin in former geological periods; the constitutive elementary granules of the cell have to-day their representative analogues in the primary micro-organisms, but they have existed in the cell from the epoch of origin, and are so adapted to social life that they no longer can become autonomous organisms.

As the brothers Zoja [1] have justly observed, this doctrine of Altmann fully coincides with the plastidular doctrine first formulated by Maggi. However, the Zojas, in their accurate study of the fuchsinophile plastidules, came to the conclusion that these have in the cell the signification of nutritive granules.

In my opinion, when the fuchsinophile plastidules have lost the exclusive value of the sole and true elementary organisms, the way to another doctrine still remains open, that of the fibrillar structure of the protoplasm, which is now acquiring a large foundation of facts.

Be this as it may, the last word has not been said on the plastidular or bioblastic doctrines of Maggi and of Altmann; such doctrines, recalling us to the study of the morphological and chemical constitution of the protoplasm, stimulate us to try to solve one of the fundamental problems of contemporary biology.

[1] L. ed R. Zoja, "Intorno ai plastiduli fucsinofili (Bioblasti dell' Altmann)," 'Memorie dell' Istituto Lombardo,' vol. xvi, 1891.

CHAPTER IV.

COMPARISONS BETWEEN THE PRESENT DOCTRINE OF PATHOGENIC MICROBES AND OTHER OLDER DOCTRINES.

THE doctrine of pathogenic microbes has not suddenly issued from the brain of a modern scientist, as one might imagine reading certain manuals published in these latter years. If we, on the contrary, cast a glance through the course of history, we nearly always find that great discoveries were preceded by vague and indeterminate intuitions, by the results of popular experience, by numerous tentatives more or less scientific, which have failed in their scope.

We can say that before every great discovery a mythical period exists in which the natural phenomenon is explained only by the genial and unconscious divinations of the poets, or by the more or less indefinite empirical experience of the people. Subsequently, when science begins to possess some means that give hope of the explanation of the unknown phenomenon being arrived at, there are numerous tentatives which aim at giving a positive and determinate foundation to this or that popular belief. These first tentatives are frequently fallacious, because the means employed are not adequate to the difficulties of the investigations, or because the problem is very complex and requires to be analysed in its elements.

Finally, when science can make use of sufficient means, when men of genius have found the method of analysing the unknown phenomenon, then the truth is discovered. As a rule such discovery is not the work of a single individual. The first discoverer sometimes finds only the path to be followed in the quest for a new truth, or he reveals new facts relative to a given problem in their *ensemble* without specifying in a definite way its conditions and particulars. The task of completing the work begun, of discovering the laws

of the phenomenon revealed by others, falls upon other men of genius. When the new principle has obtained its credentials, when its laws have been clearly defined, then it becomes a patrimony of science and of all the studious; many, fatiguing themselves over it, confirm the phenomenon of its laws with new details, and obtain useful applications from it.

Thus it has happened also for the doctrine of the pathogenic microbes.

From the most remote times, in connection with the indeterminate and contradictory ideas on effluvia, miasms, contagions, virus, the suspicion that the mysterious cause of contagious and epidemic diseases must be sought in living entities has flashed through the minds of the vulgar and the learned.

The poet Lucretius has written,—

> "Obnoxia cuncta putrori corpora
> Putrores insecta animata sequuntur."
>
> 'De rerum naturâ,' lib. vi.

Terentius Varro,[1] in his treatise on agriculture, indicated in a very precise way the existence of small beings capable of producing grave diseases. He, in fact, wrote, "*Si qua sunt luea palustria crescunt animalia quædam minuta, quæ non possunt oculi consequi, sed per aëra intus in corpus per os et nares perveniunt atque efficiunt difficiles morbos.*"

Lucius Moderatus Columella,[2] in his writings 'De re rusticâ,' also records the idea, certainly very popular in his time, of the living nature of miasms and contagions.

Although precise knowledge is wanting, still it may be considered certain that the idea of *contagium vivum* was not extinguished even in the darkness of the Middle Ages. Indeed, in a book written by St. Hildegard, doctress and abbess of the twelfth century, we find notices of minute animals which produce diseases.[3] Among others it appears that the learned nun knew also the acarus of scabies. Her book, as Haeser[4] has justly observed, has great importance,

[1] Terentius Varro, 'De re rusticâ,' lib. i.

[2] L. M. Columella, 'De re rusticâ,' lib. i, cap. v.

[3] Sanctæ Ildegardis, 'Subtilitatum diversarum naturarum creaturarum,' lib. ix.

[4] Haeser, 'Geschichte der Medicin und der epidemischen Krankheiten,' vol. i, p. 640, Jena, Dufft, 1875.

inasmuch as it summarises the popular medicine of her time.

The doctrine of contagion was not, therefore, unknown to the ancients, and even in the Bible we find that Moses has prescribed opportune rules against the propagation of contagion: "*Omni tempore, qui leprosus est et immundus solus habitabit extra castra.*"

The same principles were well known to the Genoese and Venetian navigators of the Middle Ages, who, to prevent the importation of bubonic plague from the East, from the fourteenth century had erected lazarettos, and had established very minute regulations to prevent the introduction and propagation of contagious pestilences.

The daily experience during the great epidemics of the Middle Ages had fixed in the minds of the Italians those principles, which in this century only have been accepted by science.

Boccaccio, describing the plague of Florence, has observed that "not only living with the patients gave the healthy the disease or the cause of common death, but even *touching the clothes and any other thing which the patients touched or used seemed to cause the infirmity to be transported to the toucher.*"

At the beginning of the Renaissance the ancient doctrines were revived, and received notable impulse.

Gerolamo Fracastoro [1] formulated in a very rigorous way the principle of the specificity of the contagions, and the laws of diffusion of pestilences.

Fracastoro's work on contagions and contagious diseases remains classic, and even in this century the Italian school of contagionists have taken the name from Fracastoro. In that work we find clearly indicated the *seminaria contagionum*, and in another old volume by De Bonagentibus,[2] as well as in many books on the plague published in the sixteenth century,[3] we find the *germs* of plague, the *pestis semina*, etc., mentioned.

[1] 'Hieronymi Fracastorii Veronensis is operum,' Venetiis, Juntæ, 1555, "De contagionibus et contagiosis morbis."

[2] De Bonagentibus, 'Decem problemata de peste,' Venetiis, 1556, p. 10.

[3] Francesco Alessandri, 'Trattato della peste et febbri pestilenti,' trans-

The idea of living contagions, which had met with so much favour among the ancient Romans, was therefore revived.

Even Paracelsus,[1] in the midst of his abstruse astrological and alchemistic metaphysics, seemed to have an idea, though obscure, of living contagions when he spoke of the *seeds* of diseases.

But the first who sought to give an objective, even if somewhat coarse basis to the old hypotheses and the ancient postulates, was without doubt Athanasius Kircher in 1641. Father Kircher[2] then wrote, "It is so true that the air, the water, the earth, swarm with innumerable very minute insects, that now-a-days it can be proved by the human eye. Up till now it was universally known that worms are produced in putrefying bodies; but only since the wonderful invention of the microscope has it been possible to recognise that all putrefying substances teem with an innumerable swarm of beings invisible to the naked eye: I myself would never have believed this if I had not acquired the conviction of it by experiments continually repeated in the course of many years."

In reality Father Kircher, by means of a very primitive lens, must have seen only tiny worms or tiny larvæ, or perhaps some infusorium. His discovery in any case was in itself and by itself of the greatest importance. It was received with great favour by all the intellectual world of his time, all the more because he himself drew from it the most daring consequences with regard to the etiology of diseases.

As is known, in the year 1656 the plague raged in Italy, and Father Kircher immediately proceeded to search for in

lated from Latin into Italian by the author; Turin, De Grandi, 1586, lxvi, b. 14.

[1] Paracelsus, 'Opus paramirum,' and other writings.

[2] Athanasii Kircheri e Soc. Jesu, 'Scrutinum physico-medicum contagiosæ luis quæ dicitur pestis, quo origio, causæ, signa, prognostica pestis nec non insolentes malignantis naturæ effectus, qui statis temporibus, Cælestium influxuum virtute et efficacia tum in elementis tum in epidemiis hominum animantiumque morbis elucescunt, una cum appropriatis remediorum Antidotis nova doctrina in lucem eruuntur. Cum præfatione D. Cristiani Langii, professoris medici in Acad. Lipsiensi,' publ. Lipsiæ, anno 1671.

the blood and in the pus of those suffering from plague the tiny worms which must have been the cause of the disease; and, in fact, his expectation was confirmed by the discovery of innumerable corpuscles. It appeared then to all that the doctrine of living contagion had found its confirmation in fact. Perhaps the supposed tiny worms of Father Kircher were not the products of his imagination and of the faith of his admirers; it is probable that he had seen only the blood-globules and purulent corpuscles, which at that time were not known, and that he had taken them for worms.

As Löffler[1] has well observed, the impression made by Father Kircher's books must have been very great, since Christian Lange,[2] in 1671, had them reprinted in Germany, and in an enthusiastic preface recommended them to studious youths. In that preface Lange referred also to his own ideas and observations on the genesis of epidemic purpura which developed at that time in puerperæ. According to Lange and Hauptmann,[3] this disease was to be attributed to a putrefaction of the retained lochiæ, determined by tiny worms. In the same way Lange maintained that tiny living entities were the cause not only of measles, smallpox, petechial fever, but also of certain pleurites, of certain diseases of the stomach and of the intestine. Lange,[4] in fact, wrote, "Morbillos utpote ab infami hâc putredine scatuiriginem suam adspicientes esse *animatam ac innumerorum ad instar insensibilium vermiculorum pullulaginem astruere non trepidemus.*"

Many other contemporary authors shared these opinions; indeed, we find that Zacutus Lusitanus[5] (Abraham Zacut) and the Spaniard, Juan Thomas Porcell, stated that they had observed tiny worms in smallpox and in diverse cutaneous diseases.

[1] Löffler, 'Vorlesungen über die geschichtliche Entwickelung der Lehre von den Bacterien,' Lipsiæ, 1887.

[2] Lange, Preface to the 'Scrutinium physico-medicum' of Kircher.

[3] Hauptmann, "De viva mortis imagine," 'Ephemerides naturæ cur o-sorum,' cited by Vallisneri, 'Opere,' tom. ii, p. 27.

[4] Christiani Langii, 'Miscellanea curiosa medica,' Lipsiæ, Götzenii, 1666, p. 124.

[5] Zacutus Lusitanus, 'Opera omnia,' Lugduni, 1649.

Ziengler also believed that he had found tiny worms in petechiæ, and he therefore maintained the doctrine of animated pathology. Paullini[1] expressed the same ideas in his monograph on the genus *Canis*.

But certainly the doctrine of living contagions could not obtain in the seventeenth century a favourable reception from every physician and naturalist, because all precise notions were wanting concerning the supposed microscopic worms of which the above-mentioned authors had spoken.

However, at the end of the seventeenth century appeared the first description, truly to be considered as such, of some beings absolutely invisible to the naked eye. Antony van Leeuwenhoek[2] must be considered as the fortunate discoverer of the world of the infinitely little.

He, by means of a microscope constructed by him in 1675, succeeded in discovering in rain water minute, very mobile entities, so very small that even Robert Hooke, the most celebrated microscopist of that time, succeeded in finding them only after fatiguing observations prolonged for several years. Also in sea water, in spring water, in various infusions, in the digestive tube of the frog and of birds, in his own fæces even, Leeuwenhoek discovered various forms of tiny beings very distinctly characterised by their movements.

The observations of the learned Dutchman, described in numerous memoirs published by the Royal Society of London, have extraordinary historical interest, especially because they are adorned with figures. These figures demonstrate in the most positive way not only that Leeuwenhoek had seen numerous infusoria, but also that he had recognised numerous forms undoubtedly bacterial.

From the examination of the whole of Leeuwenhoek's publications it results that he had recognised not only motile and non-motile bacilli, but also spirilla, and even micrococci. Extremely interesting are this author's observations on the movements of the discovered micro-organisms; movements which are described with such clearness, with such vivacity

[1] Paullini, 'Cynographia curiosa,' Norimbergæ, 1685.

[2] Leeuwenhoek, 'Arcana naturæ detecta ab Antony van Leeuwenhoek,' Delphis Batavorum, 1695.

of colouring and of comparisons, that they give a most exact idea of the phenomena observed.

The wise and fortunate discoverer, however, did not wish to express any judgment on the signification of the beings seen by him, nor on their importance in the economy of nature. He, like the happier discoverers, was extremely prudent, and limited himself to demonstrating with the greatest exactness the reality of his great discovery. But this discovery could not fail to be connected with the doctrine, never extinct, of animated pathology. The small microscopic beings with which the whole world occupied itself at that time, as is proved by a publication of the physician Elsholz,[1] were immediately taken into consideration for explaining the genesis of numerous diseases.

The subsequent discovery of the acarus of scabies, repeated in 1687 by the researches of Cosimo Buonuomo and Giacinto Cestoni, published in a letter to Redi, powerfully contributed to confirm and to credit still more the belief in the parasitic origin of many diseases. Andry[2] was the promulgator of this doctrine in France. He affirmed that the air, water, vinegar, wine in fermentation, fermenting beer, cider, sour milk, are full of germs; he found germs in the blood, urine, in the pustules of smallpox; he thought that all the lesions in venereal diseases were determined by invisible entities, nibbling the diseased parts.

This doctrine was also supported by Hartsoeker;[3] it appeared then that one could understand why mercury had such a favourable action in the treatment of syphilis, thinking precisely that mercury exercised a poisonous action on the tiny beings. To a certain extent they had already divined the doctrine of antiseptics and of bactericides.

In Italy, as is readily understood, the animated pathology had at that time numerous and able followers. Besides Redi, Buonuomo, and Cestoni, we must also mention Bocconi, Father Lana, Lancisi, Vallisneri, and Bianchi.

[1] Johannes Sigismundus Elsholtius, 'Ephemerid. natur. curios.,' dec. 1, anno 9, 1679.

[2] Nic. Andry, 'De la génération des vers dans le corps de l'homme,' Paris, 1700.

[3] Nic. Hartsoeker, 'Extrait critique des lettres de M. Leeuwenhoek,' à a Haye, 1730.

Bocconi[1] admitted that the plague was caused by invisible poisonous insects, capable of diffusing themselves in the air, and he suggested destroying them by fire and strong vinegar; Father Lana[2] affirmed that he had seen very minute worms in corrupt or infected blood.

Lancisi,[3] in his admirable book on the noxious exhalations of marshes, endeavoured to prove that the effluvia were formed by the products of vegetation, but he attributed a fundamental part to animalcules, invisible to the naked eye, raised from the marshes into the surrounding air, and capable of entering the circulatory current of man.

Vallisneri,[4] to whom we owe so many very precise researches on the generation of man and of animals, did not hesitate to admit that the contagious diseases were due to tiny worms; Bianchi, another celebrated physician of the seventeenth century, also held a similar opinion.

Groffon,[5] studying the plague of Marseilles in 1721, believed he had found tiny worms invisible to the naked eye in the buboes; and his contemporary Lebegne[6] also maintained that the plague of Marseilles and of Toulon was due to a verminous seed.

The great Linnæus[7] likewise, though he was not practised in the *technique* of the microscope, and, furthermore, had an extraordinary diffidence against all the observations made with that instrument, though he had accumulated in his *Systema Natura* all the world of the infinitely little into the genus *Chaos*, still had a true and able divination of the living causes of the infective diseases and of fermentations.

He, in fact, in connection with the Chaos infusorium, admitted very minute beings, perhaps not yet discovered or

[1] Bocconi, 'Osservazioni naturali,' etc., terza oss., p. 60, cited by Vallisneri.

[2] Father Lana, 'Prodromo dell' arte maestra,' cap. viii, p. 249.

[3] Lancisi, 'De noxiis paludum effluviis tractatus,' lib. i, pars 1, cap. xviii.

[4] Vallisneri, Antonio, 'Considerazioni ed esperienze interno alla generazione dei vermi,' Venezia, 1710. See also Vallisneri, 'Opere,' Venezia, Coleti, 1733, tom. ii: "Dei vermi pestilenziali."

[5] Groffon, 'Observations faites sur la peste de Marseille,' 1721.

[6] Lebegne, 'Au pestis Massiliensis e seminio verminoso,' 1721.

[7] Linnæus, 'Exanthemata viva,' Upsala, 1757.

not sufficiently studied, which were the cause—(1) of the contagions of the exanthematous diseases; (2) of the hot and violent fevers; (3) of venereal diseases. In the same category were included the organisms discovered by Leeuwenhoek, the motes that in the spring appear suspended in the air, the germs which produce fermentation and putrefaction.

Nyssander, a pupil of Linnæus, maintained a thesis, also entitled *Exanthemata viva*, in which he proclaimed that the infinitely little are the immediate agents of the diseases which are observed in the human species.

At that epoch almost all diseases were by many attributed to the action of very small animalcules; numerous epidemics were described in which either tiny worms or entozoa represented the most important causal element.

But this doctrine, at the beginning of the eighteenth century, also found powerful adversaries in the Viennese clinician, De Haen,[1] and in the English clinician, Musgrave; there were not wanting, however, even in Vienna itself, able supporters, and among these the physician Plenciz deserves special mention.

Plenciz[2] repeated the observations of Leeuwenhoek, and on the basis of the same he developed with great acumen the microbic doctrine of infective diseases, that of fermentation and of putrefaction. He established the foundations of the parasitic theory, developing the considerations which later on formed one of the glories of Henle. He, in fact, maintained that the *principium seminale verminosum* only can explain in a satisfactory way contagiosity, the specific nature of contagion, its rapid increase in the organism, the reproduction of the infecting material, its diffusibility by means of the air and of objects, and the stage of incubation characteristic of the contagious diseases. He had also a conception of the specificity of the morbid agents which rivals the doctrines of Henle and Koch; indeed, he affirmed that every disease has its special seed: just as from the seed of a plant a similar

[1] De Haen, 'Opera omnia,' Neapoli, 1780.

[2] Plenciz, 'Opera medico-physica in 4 tractatus digesta quorum: I, contagii morborum ideam novam una cum additamento de lue bovina anno 1761 epidemice grassante sistit; II, de variolis; III, de scarlatina; IV, de terræ motu,' Vindobonæ, 1762.

plant is always produced, so from the seed of scarlatina a scarlatina only is always produced; in the same way from the contagion of smallpox, smallpox is always produced. He had also an idea of the variability of the infective diseases even more complete than that expressed by Koch in his earlier works, and more conformable to the views of the most modern bacteriologists. He recognised exactly that the constitution of the patient and the conditions of place, of environment, of season, influenced the course of the disease; that is, he explained up till then, in 1762, the causes of the particular characters of epidemics.

Plenciz had divined even the doctrine of toxic products, not only concerning the tiny worms, the producers of diseases, but also concerning the germs of putrefaction. He said textually, "*Tunc aliquod corpus putrescere, quando seminium verminosum animari, evolvi et multiplicari incipit; hæc enim animalcula egerunt multa excrementa sale volatili constantia, item multa alia semina seu ovula ulteriori evolutioni inservientia, ex quibus quæ liquida sunt, turbida et male olentia redduntur, solida vero plus minus male olentia, mollia et friabilia fiunt.*"

The doctrine of *contagium vivum* then found its best champion in the very city where De Haen taught, and in France it was supported by Réaumur; and in England, the country of Musgrave, it inspired the remarkable works of Pringle[1] on hospital gangrene and jail fever, and the first researches on septic and antiseptic substances.[2]

The works of Sauvage on nosological classification, the systematic observations introduced into medicine by the so-called Physiological School of Broussais, much dimmed the star of the animated pathology; still this star was not extinguished, as we find that the Lombard clinician Giovanni Rasori,[3] in his disquisitions on stimulus and counter-stimulus, maintained that the contagious fevers were generated by invisible animalcules.

Rasori said, "I have held the opinion for many years that

[1] J. Pringle, 'Observations on the Nature and Cure of Hospital and Jail Fevers.' Lond., 1750.

[2] Id., 'Philosophical Transactions.' Lond., 1750.

[3] Rasori, cited by Bassi, 'Discorsi sulla pellagra,' etc.

intermittent fevers are produced by parasites, which renew the access during the act of their reproduction, that follows more or less quickly according to their different species."

Rasori's doctrine was accepted and defended with great ardour by all his disciples. But also other studious Italians of that time, if they did not go so far as to attribute the plague, typhoid, and other transmissible diseases to tiny parasites, all, however, had a precise idea of contagion and of its specificity.

In this connection, Michele Rosa [1] and Valeriano Luigi Brera [2] deserve mention. The latter, who also had a long experience on parasitic worms in verminous diseases, demonstrated that the idea was too coarse of comparing the action of the contagions with that of ordinary worms; and through this he maintained that the contagions were due to an organic agent much more minute, and of a much more complex action.

The same order of ideas were held by the famous clinician Borsieri,[3] who wrote, "That which contaminates, which passes here and there, which arises in diseases, spreads to those near, and generates contagion, is a special miasm that has the power of multiplying and expanding itself like a ferment."

These ideas were widely diffused in Southern Italy, and were shared by able men like Cotugno.

Scuderi,[4] the Sicilian clinician, maintained that "smallpox, phrenetic fevers, epidemic angina, parotid disease, dysentery, phthisis, whooping-cough, like measles, roseola, Hungarian and petechial fevers, and all the contagions, in short, do not arise spontaneously, but are due to specific germs or transportable *seeds*; and that, in consequence, subjecting all these diseases to the same sanitary regulations to which smallpox is subjected, they with as much facility could be extinguished and extirpated from Europe, which has for many centuries

[1] Michele Rosa, 'De epidemicis et contagiosis,' Pavia, 1782.

[2] Valeriano Luigi Brera, 'Dei contagi e delle cure dei luo effetti,' Padova, 1819.

[3] Borsieri, 'Institutiones medicinæ practicæ,' Mediolani, 1785, parag. 349.

[4] Scuderi, 'De variolis, morborumque contagiosorum origine, causa,' etc., Napoli, 1789.

improvidently received and cultivated in its breast their destructive and homicidal *germs*."

The celebrated Viennese clinician, Hildebrand,[1] in his work on contagious typhus (exanthematic typhus), also compares, in a very precise way, the unknown agent of this disease to a vegetable or animal germ. But it was especially in Italy where the doctrine of contagion, taken up by Rosa and Brera on the lines of Fracastoro, was subsequently developed and diffused by Guani, Rubini, Giannini, Pucinotti, etc.; thus when cholera appeared in Europe it was the Italians who demonstrated its contagious nature, and among the first were Tommasini,[2] Balardini,[3] Brescia, Brera,[4] Pirondi,[5] and others.

In a particular mode the disciples of Rasori, such as Pirondi, Fossati, Grassi, Bassi, etc., very numerous in Northern Italy, supported the *parasitic nature of the contagions*.

Acerbi[6] makes a minute comparison between the characters of the contagions and the characters of living beings, observing that contagions reproduce themselves, maintaining constant their specific characters exactly like organised beings.

And Grassi[7] wrote, "The hypothesis which approaches the truth, which lends itself more readily to the explanation of all the phenomena special to the contagions, and which gives sufficient reason to the attitude that some bodies have of preserving and transmitting themselves, is that which attributes the contagions to living bodies, and especially to some species of insects or worms which reproduce themselves in particular circumstances, which, being parasites according

[1] I. V. v. Hildebrand, 'Ueber den ansteckenden Typhus nebst einigen Winken zür Beschränkung oder gänzlichen Tilgung der Kriegspest und mehrerer anderen Menschenseuchen,' Wien, 1810, p. 116.

[2] Tommasini, 'Sulla contagiosità del cholera: sul cholera morbus nozioni storiche e terapeutiche,' Parma, 1831.

[3] Balardini, "Rapporti sul cholera morbus," 'Annali Univ. di Med.,' 1831 et seq.

[4] Brera, 'Prova medico-legale della contagiosità del cholera,' Venezia, 1836.

[5] Pirondi, 'Sulla contagiosità del cholera morbus,' Massiglia, 1856.

[6] Acerbi, 'Dottrina del morbo petecchiale,' Milano, Pirotta, 1821.

[7] Grassi, 'Risposta ai sette quesiti concernenti, etc., al Congresso per le quarantene del 1839,' Pistoia, Cino, 1843.

to their nature, place themselves upon human bodies, and there propagate till, after a certain time, they abandon them, or cease to multiply, or become exterminated and destroyed by circumstances contrary to their preservation; or their germs only remain, which subsequently give rise to other contagious epidemics directly the predisposing causes of these epidemics reunite.

"This doctrine of contagions caused by germs is corroborated by the observation that all the epizoötics are derived from insects or worms.

"Whatever may be their origin, it is very probable that the productive cause of a contagious disease consists in a specific organised substance, which is capable of maintaining and reproducing itself according to the laws of all beings endowed with life."

But he who best of all supported the parasitic nature of the contagious diseases of man was without doubt Agostino Bassi, of Lodi, who, fortified by his discovery of *muscardine* of the silkworm, guided by a marvellously practical sense, in 1846 was able to write: "While it was and is believed by very many, not to say almost all of the learned, that the contagions are substances of a special kind, pernicious and poisonous, and it is generally believed *canine rabies* especially is of such a nature; this terrible contagion must have been the first, in my opinion, to make us suspect that the frightful disease does not really consist of a dead substance, but of a living substance,—that is, of parasitic beings, animal or vegetable, it having been observed that the substances most capable of destroying the life of both animals and vegetables also immediately extinguish canine rabies when they are used with necessary judgment." [1]

If Bassi, who became almost blind at a young age, was not able to continue the use of the microscope and add new discoveries of microparasites to those already completed on muscardine, he, however, was able to convince himself of the parasitic nature of many diseases on the basis of clinical criteria.

[1] A. Bassi, 'Discorsi sulla natura e cura della pellagra,' etc., Milano, Chiusi, 1846.

In fact, he wrote: "Observation and experiment demonstrate to us that all the contagions disappear or cease to act in the individual whom they assail, using agents or means capable of destroying the life of the animal and vegetable beings of the lowest classes, the producers, so to speak, of contagious disease.

"Hydrophobia, syphilis, Arabian smallpox, Asiatic cholera, and other contagions caused by the work of parasitic beings, disappear or cease to act with the use of substances or agents capable of killing these parasites, the authors of the bad disease, without destroying the life of the beings which contain them." [1]

And here Bassi gives a long series of judicious counsels on parasiticide treatments, suggesting the mode of preventing hydrophobia, syphilis, smallpox, cholera, indicating how the diffusion of diseases may be avoided by means of vaccine, teaching the disinfection of wounds, the prevention of suppurations, etc.

In Italy, therefore, the large majority of practitioners rendered public opinion favourable to the doctrine of the contagions, even when it was not accepted in the other countries of Europe.

At the International Congresses it was always the Italians who tenaciously combated for the doctrine of contagion. While the Paris Congress of 1851, although directed by contrary auspices, had to listen to the courageous affirmations of Betti, Carbonaro, and Cappello,[2] in Italy Filippo Pacini [3] prepared an anatomo-pathological and etiological memoir in which he described the microbes of cholera, to which he gave the name of *vibrioni colerigeni*, illustrated the grave alterations of the intestine, the necrosis and detachment of the epithelium, produced by the enormous quantity of the vibrios developed in the same.

If the French and the Germans contend for the glory of

[1] A. Bassi, 'Dei parassiti generatori dei contagi e rispettivi rimedi,' Lodi, Wilmant, 1851.

[2] Cappello, 'Cenni storici sul sanitario Congresso internazionale aperto in Parigi il 23 Luglio, 1851,' Geneva, 1852.

[3] Filippo Pacini, "Osservazioni microscopiche e deduzioni patologiche sul cholera asiatico," 'Gazzetta medica di Firenze,' 1854.

the discovery of the anthrax bacillus, going back to the old observations of Pollender and of Rayer, it is just that the Italians should not forget the work of Pacini, who was the first to recognise a specific bacterium in a disease of man.

Remarks on the Doctrine of Spontaneous Generation.

At this period the doctrine of spontaneous generation was revived, which in the seventeenth century had been contested and proved fallacious by Francesco Redi as regards insects.[1]

But Needham,[2] towards the middle of the past century, took up the question on the basis of his researches on the appearance of germs in sprouting grains, and in vegetable or animal infusions previously subjected to boiling. By this cooking Needham maintained that he had destroyed "all the ova" of the germs pre-existing in his infusions, and had in consequence proved spontaneous generation.

This doctrine was received with great favour in France by Buffon[3] and others; in Germany it was accepted by Wrisberg,[4] Gleichen,[5] and O. F. Müller[6] himself, the great Danish observer, who was the first to describe with extraordinary exactness the morphology of bacteria, and to give a classification of them.

The discovery of the so-called green matter of Priestley,[7] which develops in infusions under the influence of the sunlight, seemed to be a new support to Needham's doctrine.

But, however much such doctrine responded to the postulates of natural philosophy, perspicacious minds were not wanting which recognised how incomplete and defective

[1] Francesco Redi, 'Esperienze intorno alla generazione degli insetti,' Firenze, 1688.

[2] Needham, 'Observations upon the Generation, Composition, and Decomposition of Animal and Vegetable Substances,' London, 1749.

[3] G. L. Le Clerce Comte de Buffon, 'Histoire naturelle générale,' Paris, 1769.

[4] Wrisberg, 'Observationum de animalculis infusoriis,' etc., Goettingæ, 1765.

[5] v. Gleichen, 'Ueber die Samen und Infusionsthierschen, und über die Urzeungung,' Norimberga, 1778.

[6] Otto Friedrich Müller, 'Animalcula infusoria fluviatilia et marina,' Haunaie, 1786.

[7] Priestley, 'Experiments on the Air,' vol. v, 1779.

were the experiments on which the supporters of equivocal generation relied.

In 1762 Bonnet,[1] of Geneva, criticised very severely Needham's experiments and those of his followers; later the greatest experimenter of the last century, Lazzaro Spallanzani, in a series of remarkable works conducted with marvellous objectivity, inaugurated those positive studies that have led to the present principle of sterilisations.[2]

First of all Spallanzani determined that a development of germs takes place in infusions even when removed from all contact with the air; then, thinking that "the ova" of the germs might be found not only in the materials of the infusion, but also on the sides of the tubes or in the contained air, he thoroughly heated the tubes, filled them with well-boiled infusions, and then sealed them when they got cold. With this test he still had a development of germs, and he thought that they must have come from the air at the moment of sealing. In fact, having filled other vessels in the same way, and having heated them for several hours in a water-bath after sealing them, he no longer had the development of germs. He had them, on the contrary, when the tubes were cracked or when the seals were broken.

On these experiments of Spallanzani a French technologist, Appert,[3] founded his method for the preservation of alimentary substances. The continuous success of Appert's method is a splendid confirmation of Spallanzani's discovery.

But Treviranus, Gay-Lussac, and others maintained that a little oxygen was necessary for the development of the germs, and that consequently one of the conditions indispensable for spontaneous generation was wanting in the experiments of Spallanzani.

Franz Schultze[4] replied to this objection, demonstrating that air can arrive in abundance in the tubes containing the

[1] Charles Bonnet, 'Considérations sur les corps organisés,' vol. ii, Amsterdam, 1762.

[2] L. Spallanzani, 'Opuscoli di fisica,' 1776.

[3] Fr. Appert, 'L'art de conserver toutes les substances animales et végétales,' Paris, 4 ed., 1831.

[4] Franz Schultze, "Resultate einer Experimentellen Beobachtung ü. Generatio æquivoca," 'Poggendorf's Annalen,' 1836.

infusions without the development of germs, when the air reaches the tubes sterilised, bubbling through sulphuric acid and caustic potash.

Schwann[1] repeated this experiment, passing the air through a metallic alloy readily fusible at about 360°.

But against all these experiments the objection was raised that the air was chemically altered by the acids, the alkalies, the heat. On the other hand, Mantegazza in a youthful work[2] described some ingenious experiments by which he had obtained the development of vibrios and infusoria in infusions of lettuce, meat, etc., boiled, reversed on mercury, and then aërated by means of a tube, which enabled the air to pass through a solution of caustic potash.

These experiments certainly responded to an able conception, but one understands how owing to their complication, and the different manipulations which they required, they very easily gave rise to contamination of the sterilised material.

Subsequently Schroeder and v. Dusch[3] showed that it was sufficient to close the tubes containing the infusions with a plug of cotton wool, and then sterilise them as Spallanzani had done. The air had free access to these tubes, but it filtered through the cotton wool, and did not give rise to the development of germs.

These experiments did not convince Pouchet,[4] who tried to demonstrate the development of germs even in sterilised liquids, and a long series of authors of great reputation, such as Mantegazza,[5] Balsamo Crivelli,[5] Oehl,[5] Cantoni,[5] Maggi,[5] Cavalleri,[5] in Italy; Joly[6] and Musset,[6] besides Pouchet, in France; Wymann[7] and Bastian[8] in England, proved that the vibrios really persisted in boiled liquids. In this almost

[1] Schwann, "Versuche ü. die Weingährung und Fäulniss," ibid., 1837.

[2] Mantegazza, "Ricerche sulla generazione spontanea," 'Giornale dell' Istituto Lombardo,' 1852, vol. iii, p. 466.

[3] Schroeder and v. Dusch, "Ueber die Filtration der Luft in Beziehung auf Fäulniss und Gährung," 'Ann d. Chemie,' 1854.

[4] Pouchet, 'Hétérogénie, ou Traité de la génération spontanée,' Paris, Baillière, 1859. Id., 'Nouvelles recherches sur la génération spontanée,' Paris, Masson, 1854.

[5] See a series of notes communicated to the Istituto Lombardo, 1865–79.

[6] See a series of notes in the 'Comptes rendus,' 1860–63.

[7] Wymann, 'American Journal of Science,' 1867.

[8] Bastian, 'Ctrbl. f. med. Wissenschaften,' 1876–8.

the whole of these authors found a confirmation of the doctrine of spontaneous generation; Oehl and Cavalleri, however, declared themselves *panspermists*, that is followers of the doctrine formulated by Spallanzani.

These studies have had great influence on the development of microbiology, inasmuch as they have led to the demonstration of the durable spores and of their extraordinary resistance.

In 1860 Pasteur[1] found that the ordinary air could be admitted to sterilised liquids without contaminating them, provided that the air enters the recipients containing these liquids through a long curved tube in which all the suspended germs are deposited, falling by the law of gravity together with the dust. And later on Tyndall[2] observed that in a sterile recipient, open, and communicating with the air by means of a curved tube, the development of germs did not take place when the air contained in the recipient itself did not contain suspended corpuscles demonstrable by the test of the incident ray.

Against the doctrine of spontaneous generation van der Broek,[3] in 1857, showed that many fermentescible and putrescible substances remain unaltered when they are received with certain precautions in apparatus absolutely deprived of germs.

Pasteur[4] demonstrated that beer does not ferment without saccharomycetes, that urine received in sterile vessels does not undergo ammoniacal fermentation, etc. Similar results were obtained by Burdon Sanderson,[5] Rindfleisch,[6] Klebs,[7] Roberts,[8] Lister,[9] Cazeneuve and Lyon,[10] Chiene and Ewart,[11]

[1] Pasteur, 'Comptes rendus,' 1860.

[2] Tyndall, 'Philosophical Transactions,' 1876–7. See also Tyndall, 'Essays on the Air in Relation to Putrefaction and Infection,' London, Longmans, Green, and Co., 1881.

[3] v. d. Broek, 'Annalen d. Chemie und Pharmacie,' 1860.

[4] Pasteur, 'Comptes rendus,' 1863.

[5] Burdon Sanderson, 'Quart. Journal of Microscop. Science,' 1871.

[6] Rindfleisch, 'Virchow's Archiv,' Bd. liv, 1872.

[7] Klebs, 'Arch. f. experim. Pathol.,' 1874.

[8] Roberts, 'Philosoph. Transactions,' 1874.

[9] Lister, 'Trans. of the Royal Society of Edinburgh,' 1875.

[10] Cazeneuve et Lyon, 'Revue mensuelle,' 1877.

[11] Chiene and Ewart, 'Journ. of Anatomy,' 1878.

Leube,[1] Watson Cheyne;[2] and they all came to the conclusion that without the penetration of germs from the air the development of germs in sterilised or sterile substances does not take place.

Hauser[3] made some very interesting experiments on the preservation of organs extracted from healthy animals, of normal blood, etc.

Marchand[4] and Meissner[5] made similar experiments, and succeeded in preserving for years spleens, kidneys, muscles (of animals) collected sterile, without disinfection or heating, in sterilised vessels closed with cotton wool.

In contradiction to all these researches, Béchamp[6] for many years, and precisely from 1851 to 1883 and later, combated with the most tenacious energy the doctrine formulated by Spallanzani and generalised by Pasteur. According to Béchamp, as we have already mentioned in another chapter, within the cells of all living beings exist elements of extreme minuteness, which he called microzymes. These microzymes, he says, are capable of transforming themselves into bacteria, and of giving rise to all the phenomena of fermentation and of putrefaction.

Wigand[7] maintains that by a process of "*anamorphosis of the protoplasm*" bacteria are formed from the protoplasm of diverse cells. In the same way Fokker[8] believed he saw the transformation of the elements of the blood into bacilli.

But later researches have now definitely established that the above-mentioned authors have not exactly interpreted the facts observed, and that they have sometimes mistaken for bacteria granules of milk, detritus of globules and of tissues, threads of fibrin, nuclei of leucocytes, and even the mycelium of fungi.

1 Leube, 'Zeitschrift f. klin. Med.,' 1881.

2 Watson Cheyne, 'Antiseptic Surgery,' 1882.

3 Hauser, 'Arch. f. gesammte Physiol.,' 1882.

4 Marchand, 'Naturwiss. Gesellsch. zu Marburg,' 1885.

5 Meissner, cited by Rosenbach and Flügge, 'Die Mikrorganis.'

6 Béchamp, 'Les microzymes dans leurs rapports avec l'hétérogénie, l'histogénie, la physiologie, et la pathologie,' Paris, Baillière, 1883.

7 Wigand, 'Das Protoplasma als Fermentorganismus,' 1888.

8 Fokker, 'Untersuchungen über Heterogenese,' Groningen, Noordhoft, 1898.

The most recent studies, by demonstrating the cilia of bacteria, by proving how their structure is not so simple as was up till lately believed, have now diffused the belief that bacteria are relatively elevated organisms, perhaps capable of reduction to a special genus of life, and consequently, even if spontaneous generation be admitted as a theoretical postulate, it must take place not by bacteria, but by beings much more simple.

Maggi,[1] in a note published in 1884, has interpreted his previous studies as researches tending to determine the conditions favourable or unfavourable to the life of bacteria.

And in reality the studies of Pouchet, Béchamp, Balsamo Crivelli, Maggi, Oehl, Bastian, led to the investigation as to how vibrios sometimes persist in boiled liquids or in certain antiseptic solutions. Following this conception, Cohn[2] succeeded in discovering the durable spores, and Pasteur initiated those studies on sterilisation which were afterwards perfected by Koch and his school.[3]

It has thus been demonstrated that milk is difficultly sterilised, because it frequently contains microbes with resistent spores; it has been proved that anthrax spores support for many hours a dry heat of 100° C., and that at 140° C. in a dry air they resist for three hours, while they die in half an hour in a current of steam of 100° C.

Thus were founded the general principles of sterilisation, from which many methods of cultivation of microbes and many rules for prophylaxis of the infective diseases have been derived.

Microzymes and Enzymes.

On the other hand, the studies of Béchamp, Maggi, Wigand, etc., have gradually matured the idea of the *enzymes*, which is now acquiring so much importance in physiology

[1] Maggi, "Influenza delle alte temperature sullo sviluppo dei bacteri," 'Bollettino scientifico,' 1884.

[2] Cohn, 'Beiträge zur Biol. der Pflanzen,' 1875.

[3] Koch und Wollfhügel, "Untersuch. ü. die Desinfection," 'Mitth. a. d. k. Gesundheitsamt,' 1881. Koch, Gaffky, Loeffler, "Versuche u. die verweithbarkeit heisser Wassendampfe," ibid., 1881.

and pathology. In fact, according to Maggi, the *plastidules* or the constitutive granules of the cells are primordial living elements—are *virtual micro-organisms*, subject to a continuous material exchange,—capable, therefore, of giving rise to the phenomena of fermentation. This conception is very similar to that which we have of the enzymes.

It is now known that some particles of the cellular protoplasm can survive for a certain time after the destruction of the remainder of the cell. Thus the *ferments* of digestion, pepsin, trypsin, invertin, ptyalin, etc., are only eliminated cellular protoplasm, and they frequently appear under the form of cellular detritus. Bacteria also elaborate ferments or enzymes, the study of which constitutes a new field little known to investigators.

By the name of *enzymes* are designated certain proteic substances of a highly complex chemical composition, readily alterable, capable, even in very small quantity, of acting on large masses of other substances, and of breaking them up, or transforming them into bodies with less heat-producing power than the substances from which they were derived.

Such enzymes or ferments, as is known, have great importance in the nutrition of living beings. They render soluble, diffusible, assimilable, many materials which in themselves could not be utilised by organisms. Thus, for example, through the work of the enzymes albumin is transformed into peptone, starch and cellulose are converted into sugar, fats are broken up, etc.

The higher organisms, as well as the lowest unicellular beings, require ferments to maintain their life: in the higher animals the elaboration of the ferments is effected by the work of special glandular organs; but in the unicellular beings also, which are organisms without organs, the ferments are a product of metabolism, indispensable for nutrition.[1]

All the ferments possess the highly marvellous and characteristic property of acting in small quantity, transforming relatively enormous quantities of other substances, while preserving almost to infinity their own integrity.

To a certain extent the enzymes bear themselves like

[1] Compare Flügge, 'Die Mikrorganismen,' Leipzig, Vogel, 1896, p. 195, *et seq.*

living matter; in fact, they expend their activity in determinate conditions only, within certain limits of temperature, and they are destroyed by the many agents that kill micro-organisms. Indeed, for a long time the enzymes, under the name of ferments, were confused with the zymogenic micro-organisms. Now they are clearly distinguished, since the enzymes can be separated from the organisms which have elaborated them, and, once isolated, they are capable of exercising their action in a completely independent way.

Enzymes, then, are the products of cellular activity, and if they survive for a certain time the cell which has produced them, and continue their action independently from the cell, they, however, are not capable of either reproduction or multiplication; their formation depends entirely upon the integrity of the generative cell.

But, through their independent activity, the enzymes, as Hueppe[1] has well observed, are *albuminoids*, or better, *active proteids*,—they constitute, to a certain extent, *a connecting link between dead albumin and living protoplasm.*

Thus the theoretical postulate of spontaneous generation is carried further away: its demonstration will be possible, perhaps, when the positive studies of physiological chemistry will have discovered in an exact way the constitution of living protoplasm.

[1] Hueppe, 'Naturwissenschaftliche Einführung in die Bakteriologie,' Wiesbaden, Kreidel, 1896, p. 32.

CHAPTER V.

THE DOCTRINE OF CELLULAR PROLIFERATION AND THERAPY.

THERE is no doubt that the doctrine of cellular proliferation, guiding pathology to the study of the seat of diseases, according to the conception gloriously indicated by Morgagni, has exercised an extraordinary influence also on therapy, inasmuch as it has furnished positive criteria for studying the pathogenesis of the symptoms and the essence of the morbid processes.

Formerly the physician, summoned to the bedside of the patient, had no other task than that of calming by empirical means the most prominent symptom of the disease; but nowadays in the presence of every morbid manifestation he endeavours to trace its original factor in order to remove it, and consequently to see its product disappear.

Thus also pharmacology is employed to subject to a more minute examination the action of drugs by determining in a more definite way their effects on the normal and pathological elements.

Even surgery has received a most potent impulse from cellular pathology. The knowledge of the laws which we have exposed on cellular proliferation, on the regeneration of the tissues with unstable and stable elements, has taught the surgeon the possibility of operations that in other times would have been believed to be contra-indicated by physiology itself.

Once demonstrated, for example, that the elements of the hepatic parenchyma, which are stable—that is, do not multiply themselves, in the adult and healthy individual, in pathological conditions, on the contrary, are capable of pro-

liferating to the reconstitution of an entire lobe extirpated, surgery, with Loreta at its head, has been able to perform resections of large portions of the liver for the purpose of removing tumours, or abscesses, or voluminous parasites.

The knowledge of the processes of proliferation of the smooth muscles, and of the elements of the intestinal glands, has rendered possible the modern triumphs of the surgery of the intestine.

Thus we have the digital divulsion of the pylorus, the extirpation of neoplasms of the stomach, and even resections and sutures of the intestine, as have been dared many times by Novaro, Ceccherelli, and others among us.

Another operation, whose physio-pathological possibility was demonstrated only by experimental researches on the proliferation of the connective tissue, of the smooth muscular tissue, and of the epithelia, is without doubt the insertion of the ureters in the rectum. By means of this operation Novaro has been able successfully to remove a cancer of the bladder and save the life of the patient, providing another way for the deflux of the urinary secretion.

The notions acquired on compensatory hypertrophy have permitted the total extirpation of a kidney, an operation which has been performed several times also in Italy with brilliant success.

The studies on regeneration of the bones by the work of the periosteum have indicated particular operative processes, through which it has been possible to remove a bone completely mortified by a morbid process (osteomyelitis), provided that the periosteum remains intact, which, proliferating, gives rise to the formation of new bone.

We may add that the studies up till now made on the proliferation of the elements, and on the healing of wounds of important organs, indicate to surgery the path for new ventures.

The experiments made on cicatrisation of the lung have permitted Fenger, Koch, and others to open pulmonary abscesses and completely cure the process, eventually destroying all the diseased part with the thermo-cautery. But experiments have advanced even further, and have

shown that entire lobes and even a whole lung can be extirpated. Biondi produced a local tuberculosis in the lung of a rabbit; he then extirpated the lung and obtained the cure of the animal.

The demonstration of the great proliferating power of the elements of the skin has permitted new plastic operations, by which large solutions of continuity are closed that otherwise would remain open, giving rise to a loss of substance and deforming cicatrices.

The doctrine of the proliferation and autonomy of the elements has given a new impulse to the practice of animal grafts. Since the experiments of Reverdin, the grafting of the epidermis on the surface of large ulcers to facilitate healing and impede the formation of deforming cicatrices has become an habitual practice among surgeons, and many of them have studied various technical methods for facilitating the grafting. Diaschenko has shown the possibility of transplanting large tracts of mucosa also, and he has observed that even transplanted connective tissue lives and proliferates, uniting with the young connective tissue of the diseased part.

Ollier has recently succeeded in transplanting the periosteum, which in its new position manifested its formative activity, regenerating the lost bone.

To repair cachexia thyropriva (erroneously called strumipriva), Eiselberg has transplanted small parts of a healthy thyroid and obtained a complete coalition; the implanted tissue preserved its structure, nourished by the neoformed vessels of the surrounding tissues.

We would add that experimental researches also demonstrate the possibility of the adhesion of grafts of the cornea; with this experimental pathology indicates a method by which, perhaps, in the future the use of an eye will be restored to individuals who have been deprived of it owing to various corneal lesions.

According to Barth, transplanted bones undergo a slow process of necrobiosis, and are re-absorbed, but in their place the periosteum, when it grows, elaborates gradually a new bone, so that the practical result is always the same.

MICROSCOPIC DIAGNOSIS OF NEOPLASMS.

The final scope of all our efforts, of all our investigations in the field of medicine, is the treatment and cure of diseases. It is needless for us to point out that the diagnosis is necessary before selecting the treatment. Well, the doctrine of cellular proliferation, developing our knowledge of certain pathological processes of the elements, has furnished new criteria for the diagnosis; furthermore, it has created a new branch of semeiotics,—that is to say, *clinical microscopy*.

In a particular way the doctrine of proliferation has provided important criteria for the diagnosis of morbid tissue tumours.

With regard to tumours, the methods of treatment and the possibilities of cure still have the same basis that they had fifty years ago. The extirpation of a tumour performed early is the sole means at our disposal for saving the life of the patient. A direct treatment which determines the involution of the morbid process and its cure will be possible only when we know the etiology of neoplasms. For the present, owing to the advantages gained by the study of the proliferations, we can recognise the anatomical constitution of tumours, and such knowledge has been fruitful of useful results. It, in fact, has furnished valuable elements for diagnosis, and precisely it has permitted the diagnosis of many initial forms.

We know well that so long as the extirpation of a tumour is the sole means of cure which the art can offer the patient, the operation itself should be performed as early as possible, especially in cases of malignant tumours. It is, therefore, necessary to know how to diagnosticate an initial carcinoma or sarcoma, to know how to diagnosticate it in useful time for the performance of the operation—for the results of the operation in respect to the life of the patient, in order that the operation itself will produce the least possible injury.

The analysis of the pathological proliferations, the demonstration of the rapidity with which the proliferation

develops, the proved possibility of regeneration of the neoplasm by the proliferation of the individual neoplastic elements left in the operative field, have furnished the surgeon with particular indications as to the *technique* of the operative act.

Through the effect of such cognitions surgeons have begun to extirpate malignant tumours in such a way as not to leave residue of the same within the organism, extirpating, if possible, all the diseased organ even when invaded only in part, terminating the operation in every case in healthy tissues.

In this mode the surgeon has been able, much better than formerly, to prevent the recurrence.

We have had a long personal experience on the histopathological diagnosis of tumours; we have preserved *à propos* very numerous microscopic preparations, together with the relative clinical histories, which would enable us to write here a treatise on clinical histopathology.

But considering the historical and critical nature of the theme proposed to us, it would be (perhaps we are mistaken) going beyond the argument to expose with minute particulars, and with the accompaniment of numerous figures, which we have ready in great part, the pathological histology of tumours.

We therefore limit ourselves to exposing a series of practical examples, demonstrating the amount of clinical importance that histopathological diagnosis possesses.

The advantages obtained by the histological diagnosis are so great that nowadays in a very large number of doubtful cases, relative to external surgery and gynæcology, the operator awaits the judgment of the microscope before proceeding to the extirpation of the tumour.

Such judgment can be made on small parts of the neoplasm spontaneously eliminated, or better still on little fragments excised, or extracted with a trocar or by scraping.

At other times, on the contrary, the surgeon proceeds to the histopathological examination after the operation, to control the clinical diagnosis and obtain from it a prognostic criterion.

In every case the microscopic examination should be made with the greatest solicitude. When possible one should prefer a rapid microscopic observation of the fresh tissue, and at the same time, after having well considered the piece under examination, its source, the organ from which it was taken, its relations with the tumour and with the diseased organ, a part of it is fixed in Heidenhain's liquid (saturated solution of sublimate in sodium chloride ½ per cent.). Heidenhain's liquid is much more convenient for the examination of tumours than alcohol or potassium bichromate. Alcohol, in fact, shrivels the tissues, destroys the red corpuscles, and partly dissolves the fats; potassium bichromate fixes well the protoplasms, but not the forms in proliferation, and consequently both may mislead our judgment. The pieces are left in the sublimate for not more than twenty-four hours, a few hours will be sufficient if the pieces be small; then they are placed for some hours in iodised alcohol, next in absolute alcohol; finally the inclusions are made in celloidin, or better in paraffin. In making the sections it is necessary to remember the position of the piece, especially if it be situated at the limit of the healthy and the diseased parts, and it should be sectioned transversely including both parts.

With regard to the stains, it is necessary to use both those which demonstrate the structure of the protoplasm and of the degenerative products, and those which show the processes of proliferation.

We shall now proceed to give an account of a series of differential diagnoses of tumours, that we have been able to make owing to the knowledge which we now possess of pathological proliferations.

Among *the tumours of the skin* that sometimes require a microscopic examination for the verification of the diagnosis, *cancroid* deserves the first place. We have seen a case of ulcerative cancroid which by a colleague was diagnosed as a callous ulcer; and in order to verify the diagnosis we extirpated a piece to submit it to microscopic examination.

A callous ulcer, as is known, presents thickened mar-

gins and bottom without the least tendency to proliferation. On microscopic examination one finds a tissue very rich in elastic fibres, necrotic on the surface, and highly infiltrated with polynuclear leucocytes. Cancroid also when ulcerated may be very hard, especially at first, and thus simulate to a certain extent a callous ulcer; but on microscopic examination the differential diagnosis is very easy. In the sections are found large wedges of flat epithelial cells, polymorphous, with frequent karyokineses, often irregular,—that is, anaplastic. Between the wedges nests are also found, formed of flattened cells superimposed like the coats of an onion, which, towards the centre, frequently undergo a corneous metamorphosis, and constitute the so-called epithelial pearls.

From the examination of the deeper wedges, from the number of the forms of cellular proliferation, and the greater or less anaplasia of these forms, one obtains an idea of the greater or less tendency of the neoplasm to spread, etc. etc.

The diagnosis of *molluscum contagiosum* is very easy. In its first stages within the epithelial cells of the stratum Malpighii protoplasmic corpuscles appear, which, increasing, displace the nucleus, and finally fill all the cell. Such corpuscles, well described by Bizzozero and Manfredi, are now interpreted as Protozoa.

Sebaceous adenoma, discovered for the first time by the celebrated Porta, is frequently confused with acne rosacea or with cancer. The microscopic examination, demonstrating the typical proliferation of the sebaceous glands, enables the diagnosis to be established in the most positive way.

Fibromata and *lipomata,* if they are not recognised by their simple clinical characters, can be readily diagnosticated by microscopic examination, observing their structure, which has its paradigm respectively in the connective tissue and in the normal adipose tissue.

On the other hand, the diagnosis of *sarcoma* is sometimes difficult. We have seen an ulceration of the middle of the leg corresponding to the crest of the tibia, of whose interpretation many doctors were very doubtful and of opposite

opinions. In a small piece extirpated from the deepest and fleshy part of the ulcer a tissue was found of the embryonic connective-tissue type, infiltrated with polynuclear giant-cells, but chiefly constituted of roundish and fusiform cells with very abundant forms of nuclear proliferation, for the most part anaplastic. After the amputation the diagnosis of sarcoma was confirmed, which had originated in the medulla of the bones.

The microscopic diagnosis of certain *tumours of the tongue and of the oral cavity* is extremely important. Very frequently an initial carcinoma is neglected by the patient and by the doctor, because it is supposed to be due to the effects of a mechanical irritation caused by a broken or diseased tooth. We have observed such a case, extremely interesting; it treated of an individual who complained of a small ulceration of the tongue exactly corresponding to a carious tooth; having extirpated a very small fragment with curved scissors, we obtained preparations, which are among the most beautiful in our collection. From the epithelium of investment we saw descending into the connective tissue, slightly infiltrated with leucocytes, epithelial wedges formed of epithelial cells larger than the normal, with very numerous forms of proliferation, sometimes hyperchromatic, hypochromatic, and with various forms of karyolysis and of cellular inclusions (parasites ?). Owing to our diagnosis of cancer the patient declined being operated upon; after two months he died, and our diagnosis was confirmed on the anatomical table.

In similar cases the clinical diagnosis is often difficult, because the cancerous ulcer is infiltrated, and may present the appearance of a syphilitic or tuberculous ulcer, or even of common suppuration.

The *nasal mucosa* also frequently furnishes material for histopathological diagnosis. One often observes there papillomata, myxomata, glandular polypi, simple hypertrophies of the mucosa, with ectasia of the blood-vessels, that on microscopic examination are easily distinguished from malignant tumours. Not rarely circumscribed tumefactions of the mucosa, due to old inflammatory processes, are observed.

In such cases we have seen in the submucosa an abundant deposit of fibrin, forming coarse networks well stainable by Weigert's method.

In the *larynx* vegetations frequently occur, sometimes neoplastic, at others infective, which not rarely require a microscopic examination to verify the diagnosis. The progress made in laryngology now permits of the ready removal of small fragments for the histopathological diagnosis.

The cases that we have most frequently examined with a diagnostic scope were malignant epitheliomata, benign papillomata, and leprous nodules.

The differential diagnosis between *malignant epitheliomata* and papilloma is easily established when we bear in mind the atypical processes of epithelial proliferation which are observed in abundance in carcinoma, and are absent in benign papilloma. The diagnosis of a leprous nodule, as is known, is verified by the demonstration of the specific bacilli.

Histopathological diagnosis has acquired during these latter years much importance in gynæcology, especially as regards diseases of the external genitals, the vagina, and the uterus.

The use of partial scrapings for diagnostic purposes has entered largely into practice, and the examination of the scrapings now constitutes a chapter of semeiotic gynæcology.

In such cases the differential diagnosis between neoplasms and chronic endometritis, between benign neoplasms and malignant neoplasms, is specially important.

Carcinoma of the uterus generally develops in the vaginal portion and in the cervix. Sometimes it forms circumscribed foci; frequently it gives rise to papillomatous vegetations, which may easily be detached by scraping and subjected to microscopic examination.

We then observe a tumultuous proliferation of the epithelium, that forms vegetations with the appearance of villi supported by a connective-tissue stroma. They consist of cylindrical epithelial cells, very atypical, rich in karyokinetic

figures, sometimes normal, and sometimes anaplastic. The diagnosis of villous carcinoma is thus easily made.

At other times, on the contrary, the cancerous tumour arises from the epithelium which clothes the os uteri, and then it constitutes a carcinoma with flat epithelial cells, polymorphous, irregular, and atypical in respect to the normal epithelium.

Finally, carcinomata of the uterus may originate from the glandular epithelia (from the crypts of the cervix or of the body of the uterus), and then one observes irregular tubes containing cylindrical cells superimposed in several strata, very atypical, and undergoing a very active anaplastic proliferation. These are the so-called *destructive adeno-carcinomata.* In a more advanced period, however, the neoplasm loses its primary tubular character, and forms wedges of atypical proliferating cells that rapidly invade the surrounding tissues.

True *adenomata* of the uterus are very rare; while, on the contrary, genuine myomata—and not only leiomyomata, but also rhabdomyomata—are not wanting.

We have had occasion to observe both forms of tumours, which clinically were diagnosticated, the first as an adenoma, and the second as a polypoid tumour or papilloma.

Fibromata of the uterus very rarely require microscopic examination; frequently, however, under the name of fibromata, many tumours are classified that it would be better to call *fibro-myomata.*

The histological examination has an extraordinary importance for recognising a singular form of malignant tumour—the *deciduo-cellular sarcoma,* recently studied by Sänger, Küstner, Chiari, Pestalozza, and Resinelli.

It treats of the decidual remnants left implanted in the uterus, which vegetate and form tufts similar to the chorionic villi, with numerous very irregular anaplastic karyokineses, sometimes with polynuclear giant-cells, or provided with colossal nuclei. The exact knowledge of this tumour is very important, inasmuch as its early diagnosis furnishes precious operative indications.

Tumours of the mamma and those of the testicle are microscopically examined, as a rule, after extirpation.

In the mamma various forms of *carcinoma* are very frequently observed, easily recognisable by the wedges and strings of epithelia in anaplastic proliferation. *Sarcomata* are more rare, and are recognised by the proliferation of the connective-tissue elements. *Pericanalicular* or diffuse *fibromata* are not rarely found there.

The malignant epitheliomata are also frequent in the *testicle;* they originate from an atypical proliferation of the epithelia of the contorted canaliculi. *Sarcomata* arise through anaplasia of the connective tissue, generally from the testicle itself, rarely from the epididymis. We have sometimes observed mixed forms of connective-tissue tumours, such as fibro-sarcomata and fibro-chondrosarcomata.

CHAPTER VI.

THE DOCTRINE OF THE PATHOGENIC MICROBES AND THE TREATMENT OF HUMAN INFIRMITIES.

ALTHOUGH the doctrine of pathogenic microbes was solidly constituted only about fifteen years ago, still it has already exercised a profound influence on the treatment of human infirmities.

The advantages which this doctrine has given to the art of medicine are of multiple nature, and can be grouped into diverse categories.

We have already mentioned in the previous chapter the fundamental conception of modern therapy that requires, above all, a good diagnosis—a diagnosis which reveals the anatomical basis of the morbid process, and possibly indicates its causes. The doctrine of pathogenic microbes has given us a first advantage by indicating the causes of many diseases. By indicating the causes microbiology has taught us how to search for them in the diseased organism, and has thus furnished, by the demonstration of the specific germs within the pathological products, the precise, objective, and undeniable criteria which permit, when they are found, the diagnosis to be made with absolute certainty.

The morbid agents of many infective diseases having been demonstrated, microbiology has enabled us to study individually these agents, and to determine in what way we can prevent them invading the organism. It has thus developed an entirely new branch of prophylaxis, we may say even the principal branch that constitutes the second category of the advantages obtained from the new doctrine.

Intimately connected with prophylaxis is surgical antisepsis, which Lister and Bottini wisely derived from the

early discoveries of Pasteur, and which has permitted, in this last quarter of the century, operations so grave and so daring that no one previously would have dreamed of as being possible.

The fourth series consists in the preventive immunisations, which save men and animals from disastrous epidemics.

In fine, the fifth category of advantages consists in a new specific method of treatment for infective diseases. This specific method includes in its most recent triumphs the serum-therapy of diphtheria.

We shall now proceed to pass rapidly in review these diverse categories of advantages.

Microbiological Diagnosis.

Nowadays almost all clinicians admit that microbiology has become a science subsidiary to practical medicine, inasmuch as it, by furnishing positive data for the diagnosis, has given a more solid and broader foundation to treatment.

But the doctrine of pathogenic microbes has not furnished indications which serve the requirements of practical diagnosis for all the infective diseases; still, from the subsidies that it has given us, and which have largely entered into practice, we can already foresee that in the future it will give us even more precise data, directly scientific researches will have provided the necessary elements.

One of the greatest advantages which microbiology has rendered to diagnostics is without doubt the method of recognising the *tubercle bacilli* in the sputum and in other excretions of phthisics.

This method was introduced for the first time by Koch,[1] and was afterwards simplified by Ehrlich for the requirements of practice.

The sputum, spread out on the cover-glass, is left to

[1] Koch, "Aetiologie der Tuberculose," 'Berliner klin. Wochenschrift,' No. 15, 1882; and 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' Berlin, 1884.

dry, and then it is slowly passed three times through the flame. Then the cover is placed in a staining solution composed as follows:

Gentian violet grs. 1·50, aniline oil grs. 3·00, absolute alcohol grs. 15·00, distilled water grs. 85·00. The recipient, which contains this solution and the cover, is heated until steam develop; then the glass is passed rapidly through a solution of nitric acid 25 per 100; it is washed in alcohol, immersed for half a minute in an aqueous solution of vesuvin 1 per cent.; then it is dried and mounted in balsam. In these preparations the tubercle bacilli are stained a dark blue, while the other micro-organisms and the diverse elements of the excretion are stained brown.

The demonstration of the tubercle bacilli in sputa has the greatest diagnostic interest, especially, as Strümpel[1] has well observed, in doubtful cases or in initial forms, inasmuch as the tubercle bacilli may already be found in the sputum at a period in which all the other symptoms, and all the minutest proceedings suggested by semeiotics, would not be sufficient to enable us to make a positive diagnosis.

No less interesting is the search for the tubercle bacilli in other pathological products,—in the urine, for example, where they may be the only positive sign of a primary tuberculosis of the urogenital apparatus; or in the fæces, where they may be the index of an intestinal tuberculosis, which might be confounded with other morbid processes of the digestive tube. Nor is the advantage less for the diagnosis of the initial or doubtful forms of lupus, of anatomic tubercle, of fungus or scrofulous inflammations, etc. etc. Finally, we wish to add that the detection of the tubercle bacilli in the blood has in many doubtful cases permitted the diagnosis of acute miliary tuberculosis (Weichselbaum[2] and Lustig[3]), which with so much difficulty is distinguished from typhoid, cerebro-spinal meningitis, etc.

[1] Strümpel, 'Specielle Pathologie und Therapie.'

[2] Weichselbaum, "Bacillen in Blute bei allgemeiner Miliartuberculose," 'Deutsch. med. Wochenschrift,' 1884.

[3] Lustig, "Ueber Tuberkelbacillen," etc., 'Wien. med. Wochenschrift,' 1884, No. 48.

The bacteriological diagnosis of *diphtheria* has also acquired extraordinary importance in these latter years, inasmuch as the result of the specific treatment with the injection of the antidiphtheritic serum depends on the promptness of the diagnosis of the initial forms.

Diphtheria, as is known, in its initial phase may easily be confounded with diverse forms of angina or of ordinary and benign tonsillitis, and also with the so-called necrotic angina, etc.

For the bacteriological diagnosis it is necessary to make a microscopic examination of the exudate (pseudo-membrane), and prepare cultures. The pseudo-membranes are detached with a small sterile forceps or with a swab of cotton wool previously sterilised. Directly it is detached, the pseudo-membrane is transferred to a sterile test-tube, to make the preparations and cultures.

To make a microscopic preparation the pseudo-membrane is smeared on a cover-glass; the glass is allowed to dry, is passed three times through the flame, and placed in Weigert's liquid, already described in speaking of the tubercle bacilli. After a minute or two the glass is removed and immersed in Gram's liquid (iodine gr. 1, potassium iodide gr. 3, distilled water gr. 300); then it is dried with filter-paper and placed in aniline oil, where it is left till decolourised. From the aniline oil it is cleared in xylol, and finally mounted in balsam. In these preparations the diphtheria bacilli and the cocci of suppuration, which nearly always accompany the former, are stained an intense violet.

The diphtheria bacilli are very easily recognised by their characteristic disposition, that is by their tendency to arrange themselves in clumps or in groups, in which the individual elements or pair of elements are arranged parallel to one another.

For the cultures Löffler [1] and Roux's [2] method serves well; they suggest making the successive inoculations by stroking

[1] Löffler, "Entstehung der Diphterie," 'Mittheil. der Kais. Gesundheitsamt.,' vol. ii, 1884.

[2] Roux et Yersin, "Contribution à l'étude de la Diphtérie," 'Annales Pasteur,' 1890.

with the little piece of pseudo-membrane a series of three or four tubes of sloped solidified serum. These tubes are kept in the incubator at 37° C. for twenty-four hours; after this time the diphtheria bacilli are well developed: in the first tube the colonies are numerous and confluent; in the last tube there are a few isolated colonies.

To facilitate the isolation of the colonies, D'Espine and De Marignac [1] suggest first washing the pseudo-membrane in boric acid 2 per 100; with this one obtains the advantage of removing many accidental saprophytes, and of thus facilitating the isolation of the diphtheria bacilli.

He who is practised in this kind of study, with the naked eye can recognise the colonies of Löffler's bacillus and distinguish them from those of the streptococci.

The colonies of the latter are small and delicate, finely granular, and have under the microscope the appearance of granules of sand. The colonies of the diphtheria bacilli are whitish, umbilicated in the centre, and present an annular stratification.

The microscopic examination of the colonies in question verifies the diagnosis, demonstrating the bacilli a little swollen at the extremities, generally most stained at the poles.

For the diagnosis of diphtheria the microscopic observation of the pseudo-membrane is, as a rule, sufficient; if this research leave any doubts, cultures are made. From the innumerable clinical researches made in these latter years it results that such positive discovery is sufficient for the certain diagnosis of diphtheria. And then we must immediately adopt isolation, disinfection, and serum-therapy.

Some authors have spoken of pseudo-diphtheritic bacilli, and have maintained that they may be mistaken for Löffler's bacillus, thus removing all value from the bacteriological diagnosis. But the researches of Roux, Jersin, and Fraenkel have shown that the pseudo-diphtheritic bacilli are an attenuated variety of the Löffler bacilli, and that even in grave diphtheria the very virulent bacilli are always found associated with the attenuated bacilli. And inasmuch as

[1] D'Espine et De Marignac, "Recherches expérimentales sur le bacille diphtérique," 'Rev. m. de la Suisse romande,' 1890.

the attenuated bacilli may at any moment acquire their virulence, the demonstration of the diphtheria bacilli preserves all its practical value.

In phlegmonous and *simple anginæ* the bacteriological examination has also a notable diagnostic importance. As a rule, in simple anginæ streptococci, or staphylococci, or pneumonic diplococci (Fraenkel—Weichselbaum) are found; cases are also observed in which the *Bacterium coli commune* alone is met with.

In the more benign forms of angina or of lacunar tonsillitis the pyogenic staphylococci only are found in the substance of the purulent points. In the angina with membranous exudate, in the phlegmonous angina, streptococci, sometimes isolated at others accompanied by staphylococci, are found.

These forms of angina frequently simulate diphtheria, and the bacteriological examination is of great advantage for the diagnosis.

There are anginæ which arise very rapidly with shiverings, high fever, and then terminate by crisis. In these forms the pneumococci are met with. A mixed infection, however, occurs in a large number of anginæ,—that is to say, diverse bacterial species are found at the same time, and the clinical course is varied in consequence. The bacteriological diagnosis is made by means of the microscopic examination and cultivation as for diphtheria. The plate cultures are useful here for distinguishing the diverse bacterial species.

The *bacteriological examination in cases of pleuritis* has great importance for the prognosis and treatment. The result varies, and furnishes us with the essential criteria, demonstrating the origin of the process.

In many pleurisies with serous exudate the bacteriological examination of the liquid extracted with a syringe, according to our experience, may be absolutely negative. In many cases of pleurisy which follow pneumonia the pneumonic diplococci are found; in the pleuritic exudates that form in the course of pulmonary tuberculosis the tubercle

bacilli may be met with. Sometimes these micro-organisms are not found in the little material observed, but if the examination be positive, as is readily understood, it has great prognostic value.

In pyothorax pyogenic streptococci, or staphylococci, or pneumococci may be found. As Netter has well shown in a work rich in much clinical experience, and as we ourselves can confirm, the prognosis varies very much according to whether one has to deal with pyothorax by pneumococci or with pyothorax by the ordinary pyogenic microbes (staphylococci and streptococci); the former is much more benign than the latter, and, as a rule, gets well after the first emptying, and the successive disinfection of the pleural cavity; the latter, on the contrary, lasts for a very long time, and requires a longer intervention (operation of Estländer and Schede, etc.).

In rare cases of pleuritic exudates, which appear during typhoid fever, the typhoid bacilli are met with. In the pleurites which appear during convalescence from smallpox, staphylococci are found; streptococci, instead, are common in post-scarlatinal empyemas. In some cases of putrid pleuritis, besides the pyogenic microbes, the bacilli of putrefaction, and especially the *Proteus vulgaris*, are met with.

When we are in doubt as to a pleurisy being of tuberculous origin, and the micro-organisms are not found in the liquid extracted, it will be useful to inoculate the material in the peritoneum of a guinea-pig, to verify whether the animal will subsequently die of tuberculosis or not. Since the bacilli may be very scarce in the exudate, it will be advisable to make very abundant injections; or, better still, as we habitually do, centrifugalise a large quantity of the exudate, then decant the liquid, and inoculate guinea-pigs with the precipitate in the bottom of the tubes.

For *pericarditis* also the observations we have made in reference to pleuritis are useful. Even here a bacteriological diagnosis can be made during the life of the patient, and we thus acquire important diagnostic criteria when, in the course of the disease, we have such manifestations as permit of the pericardium being punctured.

The *bacteriological diagnosis of pneumonia* has not great clinical importance in ordinary cases, where the physical examination furnishes criteria more than sufficient. In some cases, however, the discovery of the pathogenic microbes may serve to solve some doubts.

For the bacteriological diagnosis authors have followed two methods : the one consists in the examination of the sputa, the other in puncture of the hepatised lobe of the lung.

The examination of the sputum is made in the following mode :

The patient expectorates into a sterile glass capsule containing sterilised water. Then one immediately proceeds to the quest, thoroughly shaking the dense sputum in the water to get rid of all the saprophytic germs of the mouth and fauces; then the sputum is placed in a sterile glass slide, and with sterile needles a small particle of the internal portion of the sputum is isolated.

With this particle stroke cultures are made in glycerine-agar or in broth. We have succeeded very well also with agar plates, with added human serum taken from pleuritic exudates, or with agar and blood. When these cultures are made one proceeds to the microscopic examination of another particle of the sputum, in which, with the Günther-Friedländer method, innumerable diplococci capsulati are found. For this method the cover-glasses, passed through the flame as usual, are placed for a few minutes in acetic acid 1 per 100; then they are dried with filter-paper, are left to dry a moment, are stained with Weigert's liquid (see method for tuberculosis), and finally washed in water and examined in water.

Many authors, including Bozzolo, Monti, Netter, Patella, etc., have punctured the diseased lung with a small syringe, and extracted a few drops of the exudate, with which they have made the bacteriological examination. This method offers the advantage of furnishing a pure material free from the accidental microbes of the mouth.

Such puncture of the lung, performed with the necessary antiseptic precautions, has never produced any inconvenience, and is now recommended in diverse treatises.

From the practical point of view, however, the puncture is rarely required for diagnostic purposes. As a rule the examination of the sputum is sufficient, and precisely its microscopic examination, which enables us to verify whether the process in progress be due exclusively to the pneumococcus or to the tubercle or other germs. The most frequent case is that in which one observes a sputum very rich in blood elements that makes us suspect an acute pulmonary tuberculosis. In such case the absence of the tubercle bacilli and the presence of the pneumonic diplococci establish the diagnosis.

The microscopic examination is more important than the cultures, inasmuch as the pneumococci are sometimes very difficult to cultivate, and require particular technical aptitudes. The broth cultures only are constantly successful.

When, however, the plate cultures are successful they have notable value, because they serve to identify the pneumococcus in a precise way, and they enable us to see positively whether the pneumococcus is solitary or associated with pyogenic microbes. According to Monti, See, and others, the pyogenic microbes are frequently found together with the pneumococcus in genuine pneumonia, and have a more grave prognostic signification, inasmuch as they indicate grey hepatisation and suppuration. We, however, should never give a favourable prognostic signification to the exclusive presence of the pneumococci, because the termination of the process may vary very much according to their virulence, the resistance of the individual, etc. Only in some cases shall we be able to foresee a possible resolution by crisis.

In *influenza* the bacteriological diagnosis has acquired much importance since the numerous clinical observations made especially during the epidemics which have so gravely affected Germany and Austria during these latter years. Pielicke,[1] Kruse,[2] Finkler,[3] Borchardt,[4] and others have shown that diverse diseases run with the clinical mani-

[1] Pielicke, "Bacteriologische Untersuchungen in der Influenzaepidemie," 'Berliner klin. Woch.,' 1894, No. 23.

Notes continued on opposite page.

festations of influenza,[2] and that in such cases the bacteriological examination has great importance for the differential diagnosis. Borchardt, for example, cites a case in which the diagnosis was doubtful between typhoid and grave influenza, which prevailed in Berlin in 1894; the bacteriological examination decided in favour of influenza, and this diagnosis was confirmed by the subsequent course.

For the bacteriological diagnosis of influenza Pfeiffer[5] has considered the microscopic examination and the successive cultures of the sputa to be necessary. Many authors to-day, like Kruse and Finkler, maintain that the microscopic examination is sufficient for those who have an exact knowledge of Pfeiffer's bacilli.

For the microscopic examination the sputa are collected in a sterile capsule, the preparations are made on cover-glasses, and are stained for at least ten minutes with a solution of carbol-fuchsin or, better, with methylene blue.

In the preparation the bacilli appear very small; they are perhaps the smallest known; they are rather short, and usually occur in pairs or in clumps; they are often seen in great numbers within the leucocytes. In the bronchial sputa of those suffering from influenza they are very abundant, and are sufficient for the diagnosis when by previous experience they are well known.[6]

The cultivation of the influenza bacilli takes place only at a high temperature (37° C.) and on nutritive media which contain hæmoglobin or leucocytes. If with a particle taken from the sputum, according to the rules indicated *à propos* of pneumonia, the surface of the agar or solidified serum be smeared, a development is obtained, if somewhat

[2] Kruse, "Zur Aetiologie und Diagnose der Influenza," 'Deutsche Wochen.,' 1894, No. 24.

[3] Finkler, 'Infektionen der Lunge durch Streptokokken und Influenzabacillen,' Bonn, 1895.

[4] Borchardt, 'Beobachtungen über das Vorkommen des Pfeiffer'schen Influenzabacillus," 'Berl. klin. Wochen.,' 1894.

[5] Pfeiffer, "Vorläufige Mittheilungen über die Erreger der Influenza," 'Deutsche med. Wochen.,' 1892, No. 2.

[6] Pfeiffer, "Die Aetiologie der Influenza," 'Zeitschrift f. Hygiene,' vol. xiii, 1893.

scarce, after twenty-four hours. The colonies appear in the form of tiny drops like dew; even under the microscope these colonies appear glistening, completely homogeneous, and colourless. The colonies of a few days old present a yellowish colour towards the centre; however, they never become confluent, but always remain isolated. Sub-cultures of the first culture on tubes of agar or of solidified serum do not develop if some drops of sterile blood are not placed on the nutritive medium; by this expedient only can the bacilli be cultivated for many generations, provided that the sub-cultures are made every three or four days.

For diagnostic purposes it is useful to make the cultures directly with media on the surface of which a drop of sterile blood or a solution of hæmatogen is first placed, according to Huber's[1] advice. On these media, with added hæmoglobin, the development is much more vigorous, and when the successive smearings are made on diverse tubes with the same particle of sputum, the influenza bacilli and the other bacterial species which sometimes accompany them are also isolated.

According to the German authors already cited, by careful examination one easily succeeds in finding the characteristic bacilli in all cases of influenza. In the greater number of the genuine cases a microscopic preparation is sufficient for establishing the diagnosis. In the less recent cases, or those complicated, the cultures almost always give a positive result.

The bacteriological examination in general has permitted a precise judgment in many cases clinically not well definable, and has led to the diagnosis in many sporadic cases otherwise unrecognisable.

In *ulcerative endocarditis* the bacteriological examination has been useful in many cases, but it is not of general and absolute importance. In cases of endocarditis developed after suppurative processes, during the course of puerperal infections or of other surgical infections, one may be able to observe pyogenic staphylococci and streptococci in the

[1] Huber, 'Zeitschrift für Hygiene und Infektionskrankheiten,' vol. xv.

circulating blood, and in such cases the diagnosis can be firmly established. But in many cases of ulcerative endocarditis and of malignant endocarditis (Litten's form) the bacteriological examination of the blood has been without result.

This fact is not surprising when we think that the morbid agents vegetate in such cases on the valves of the heart, from whence they pass into the circulating blood only in small number.

In the forms of *pyæmia* the demonstration of the bacteria in the blood is much more frequent, especially during the rigor, when fragments of the thrombi, loaded with the micro-organisms, are carried in the circulation, where they form infecting emboli, the generators of new metastases.

Such examination is frequently very easy even in cases of septicæmia and of cryptogenetic pyæmia. In these cases especially the bacteriological search may be of great aid for the diagnosis, inasmuch as the point of departure of the infection (which may be an abscess of the prostate, a purulent gathering in the antrum of Highmore, etc.) being unknown, the criteria for judging the nature of the disease are wanting.

The *technique* for the examination of the blood in these cases is as follows:—a finger is thoroughly washed with soap, then with alcohol and ether, to remove the grease; it is disinfected with sublimate, dried with alcohol and ether, and then its fleshy part is pricked with a sterile lancet. The drop of blood which appears is taken up in the sterile platinum loop, and finally the agar or serum is smeared with it.

Some recent authors, such as Bozzolo and Cantur, recommend the extraction of a larger quantity of blood by opening a vein of the arm, of course with the usual bacteriological precautions.

To avoid useless repetition we shall here mention the *bacteriological examination of abscesses*, which, owing to the metastases, develop in the course of pyæmia. The abscesses are opened with sterilised instruments, the skin having been previously disinfected as above described. From the pus

that discharges stroke cultures are made on the surface of agar or of sloped solidified serum, and cultures disseminated in a Petri's capsule, placing a loop of pus in a tube of agar and of liquefied gelatine, which is then poured into the sterile capsule and there becomes solidified.

A few drops of pus serve for direct microscopic preparations, which are stained by Gram's method. This method consists in placing the glasses, dried and passed through the flame as usual, in Weigert's liquid; after a few minutes the glasses are removed and immersed for a few seconds in Gram's liquid (I gr. 1, IK gr. 3, distilled water gr. 300); then they are decolourised with absolute alcohol, a second staining with eosin, are washed anew, dried, and mounted in balsam. By this method the pathogenic microbes are stained an intense violet, while the ground is stained red.

These rules, as is readily understood, are also of use for the foci of *osteomyelitis,* when the abscesses have reached the periosteum and the skin.

The same may be said of *puerperal infections,* whose grave forms most frequently are peritonitis and pyæmia. For peritonitis, when the exudate has collected in the peritoneal cavity, it is sufficient to extract a small quantity of it with the usual rules, and treat it according to the above-described methods.

The micro-organisms which are found in all these forms (which must not be confused with the specific septicæmias, due to anthrax, malignant œdema, etc.) are the so-called common pyogenic germs, whose biological characters we shall now summarily describe.

The staphylococci are divided into three species,—*Staphylococcus pyogenes aureus, albus, citreus.* These three species differ from one another only in the colour of the pigment produced in the cultures; they have the form of round cocci arranged in clumps that have been compared to a bunch of grapes, hence the name. They develop well at the ordinary room temperature, but better at 37° C. In cultures disseminated in gelatine they form roundish colonies, granular, with distinct contours, whitish at first in all three species, then golden or citrine if it treat of *aureus* or of *citreus.*

Directly the pigmentation begins the gelatine liquefies around the colonies, and the fusion proceeds with considerable rapidity. In stroke cultures made on the surface of agar or serum there is an abundant growth, glistening, golden in *aureus*, white in *albus*, citrine in *citreus*.

The streptococci, according to some authors, also consist of three species (*Streptococcus brevis*, *Str. longus*, etc.), but as the differential morphological and biological signs are very slight, and their pathogenic properties are equal, the majority of bacteriologists hold that they are only varieties of a single species.

The morphological characteristic of the streptococci is their arrangement in chains. These chains consist of round elements arranged behind one another like the beads of a rosary. They have this appearance both in the pus and in the cultures. Some varieties form chains of a few elements, others long rosaries more or less coiled on themselves. In cultures disseminated in gelatine the streptococcus forms small colonies, whitish, finely granular; the gelatine never liquefies. If in the inoculated media streptococci and staphylococci are found together, the colonies of the latter are easily distinguished from the beginning, inasmuch as they are larger and more opaque; later they are even better recognised, because they liquefy the gelatine and present the characteristic coloration—golden, or milky white, or citrine.

In some cases togther with the above-mentioned species another micro-organism, the *Bacillus pyogenes fœtidus*, is also observed, which nowadays many authors are inclined to identify with the *Bacterium coli commune*.

We shall speak of the latter in another paragraph.

As regards the prognostic signification of the diverse pyogenic microbes found, some authors, with Rosenbach [1] at their head, have believed it possible to give a diverse value to the streptococci in respect to the staphylococci, judging that the former are more malignant than the latter.

In reality the streptococci have a tendency to give rise to less destructive but more invading processes; they spread

[1] Rosenbach, 'Wundinfektionskrankheiten,' Wiesbaden, 1885.

with great rapidity, following especially the channel of the lymphatics.

The staphylococci produce circumscribed but more destructive suppurations, they easily attack the parietes of the blood-vessels, and give rise to infecting emboli. Thus, for example, among the puerperal infections pyæmic forms are observed with the production of numerous metastatic abscesses, and forms having the characters of internal malignant erysipelas (Virchow), which manifest themselves with peritonitis. The former are caused by the staphylococci, the latter by the streptococci. In many cases, however, the different species of pyogenic microbes are found associated, and then the above-mentioned differences can no longer be distinguished.

In *peritonites* the bacteriological diagnosis has a notable value, both for the prognosis and the treatment.

We know very well that there are multiple forms of peritonitis with very different etiology, in relation to which the prognosis and the treatment are very varied.

There are peritonites following perforation of the intestine in which intestinal micro-organisms, and especially the *Bacterium coli,* are met with.

There are traumatic peritonites characterised by pyogenic microbes and by diverse putrefactive bacilli. Peritonites of tuberculous origin are frequent; those due to the pneumococci are not wanting; there are even aseptic peritonites due to the absorption of bacterial toxins (or of other nature) from the intestine.

The bacteriological examination is made according to the rules already indicated in the preceding paragraphs,—that is to say, an exploratory puncture is made with the necessary precautions, and a small quantity of the exudate is extracted with which the microscopic preparations and the cultures are made.

The extraction of the exudate is easy in the greater number of cases; it is difficult only in tuberculous peritonites, and, as a rule, in the chronic forms.

When the clinical course and the microscopic examination make us suspect tuberculosis, without being able to discover

the characteristic bacilli, it will be useful to inoculate the exudate in a guinea-pig, as we have described for pleuritis.

In other cases of peritonitis the cultures will be useful for the diagnoses, demonstrating the presence of the pneumococcus in some forms of peritonites relatively benign; they will demonstrate staphylococci, streptococci, and putrefactive bacilli in traumatic peritonites, the *Bacterium coli* especially in peritonites following perforation.

In the appendix to the paragraph on peritonitis we shall note how in *perityphlitic abscesses* the *Bacterium coli commune* is always met with.

In *hepatic abscess* the bacteriological examination has still greater importance. As is known, there are hepatic abscesses in relation with echinococci, without the intervention of pyogenic microbes. There are, on the other hand, hepatic abscesses in relation with dysentery of tropical countries, and in them amœbæ and pyogenic micro-organisms are found. Other abscesses of the liver are due to angiocholites determined by the *Bacterium coli*.

Exploratory punctures, made with the precautions several times already mentioned, readily permit the extraction of a more or less abundant quantity of the exudate.

With this the microscopic preparations and the cultures are made, as we described when speaking of pyæmic abscesses. If bacteria are not found either by examination of the direct preparations or by means of the cultures, it will be necessary to search for amœbæ, or for the hooklets of the echinococcus.

Many writers on tropical pathology, such as Kartulis,[1] Councilmann,[2] Kruse and Pasquale,[3] state that amœbæ are constantly found in hepatic abscess of tropical countries when it has developed after dysentery. In the so-called idiopathic abscesses, on the other hand, the ordinary pyogenic microbes only are met with. We, however, believe that this criterion is not absolutely true; on the contrary, we have reason to

[1] Kartulis, 'Centralblatt für Bacteriologie,' December, 1887; and 'Zeitschrift für Hygiene.'

[2] Councilmann, 'Johns Hopk. Hospit. Rep.,' Baltimore, 1891.

[3] Kruse and Pasquale, 'Zeitschrift für Hyg. und. Inf.,' 1891, vol. xvi.

believe that even in some post-dysenteric abscesses bacteria alone are found. Be this as it may, it is certain that the discovery of the amœbæ when it is positive enables us to diagnosticate the so-called tropical hepatic abscess.

In the greater number of the idiopathic abscesses the pyogenic staphylococci are met with; the *Bacterium coli* also is frequently found, as Dmochowski and Janowski [1] have demonstrated. In some cases Pansini [2] has found a simil-typhoid bacillus.

The *Bacterium coli commune* in pus usually presents the form of short bacilli, almost always in pairs; in cultures, besides the short bacilli, it also forms longer, straight or curved rodlets. It stains with an alkaline solution of methylene blue or with carbol-fuchsin; it does not resist Gram's method. It grows well at the room temperature, but better at 37° C.

In cultures disseminated in gelatine it forms deep punctiform yellow colonies, and superficial colonies which extend in a glistening bluish-white growth, with lobulated margin, and with a thicker centre. If the gelatine be a little dry, it forms, on the contrary, white and raised little heads. In gelatine stab cultures there is along the stab a nodose line, and a bluish-white growth at the surface. In the gelatine itself bubbles of gas form, and sometimes crystals.

The cultures on agar form dense yellowish growths: those in bouillon rapidly become turbid, and give the indol reaction; those on potato develop a brownish thick growth, raised; those in milk determine its coagulation.

With regard to *nephrites*, many authors give great diagnostic and prognostic importance to the bacteriological finds in the urine.

As is known, normal urine never contains microbes in the bladder; during micturition only it may carry with it some micro-organisms from the last part of the urethra or from the skin. To collect the urine for bacteriological

[1] Dmochowski und Janowski, 'Centralblatt für allgemeine Pathologie,' 1894, No. 4.

[2] Pansini, 'Riforma medica,' 1893, Nos. 95, 96.

purposes it is sufficient to extract it with a sterile catheter. The urine thus obtained is immediately centrifugalised in sterile tubes, and the sediment thus formed serves for making the microscopic preparations and cultures, eventually also for inoculations in animals.

The etiology of nephrites is very varied; there are primary infective nephrites, infective nephrites associated with general infections, toxic nephrites, etc.

In primary infective nephrites, according to Mannaberg,[1] the bacteriological examination is of notable diagnostico-prognostic advantage, as in the acute nephritis which has a rapid and favourable course streptococci are ordinarily found, while these micro-organisms are absent in the nephrites that afterwards become chronic.

The *Bacterium coli*[2] has been met with in some very grave forms of acute hæmorrhagic nephritis, and in many forms of pyelo-nephritis.[3]

In other cases of primary acute nephritis the *Staphylococcus pyogenes albus*[4] has been found, and in others still the pneumococcus.

In the nephrites secondary to other diseases the pyogenic microbes are sometimes met with, at other times the germs of the primary disease:[5] thus, for example, in post-scarlatinal nephritis a streptococcus is nearly always observed in the urine; in grave metapneumonic nephritis the pneumococcus has been found; in nephritis following a typhoid infection the typhoid bacilli[6] have been met with.

[1] Mannaberg, "Zur Aetiologie des Morbus Brightii acutus," 'Centralblatt f. Bakt.,' 1888, No. 30.

[2] Jeanselme, "Note sur un cas de néphrite aiguë hémorragique, causée par le *Bacterium coli commune*," 'Gazette hebdomadaire de Méd. et de Chir.,' 1893, No. 24.

[3] Mircoli, "Forme morbose da *Bacterium coli commune*," 'Gazzetta degli Ospitali,' 1893, No. 50.

[4] Lenander und Sundberg, "Perinephritis acuta post nephritidem ascendentem gravidæ (Bacterium coli commune)," Upsala, 'Läkareförenings Förhandlingar,' Bd. xxix, 1894.

[5] Baduel, "Nefrite primaria acuta emorragica da stafilococco bianco," 'Riforma medica,' 1894, vol. iii.

[6] On tuberculosis of the urinary apparatus and the search for tubercle bacilli in the urine see the paragraph on tuberculosis.

In post-diphtheritic nephritis bacteria are not generally observed; the process appears to be due exclusively to the action of the toxins.

The streptococci which represent, as is readily understood, a secondary infection, are rarely found.

In *cystites* the bacteriological finds are much more constant, and furnish much more positive diagnostic and prognostic criteria.

On this argument we have nowadays a very rich literature. From the analysis of this literature and from our personal experience it results that the greater number of the primary acute cystites are due to the *Bacterium coli commune;* others are caused by the *Streptococcus pyogenes.* This is the rule; nevertheless cystites due to the *Staphylococcus pyogenes aureus,* to the *Urobacillus septicus,* which to-day tends to be identified with the *Bacterium coli* (Krogius), to the *Staphylococcus ureæ liquefaciens,* to the *Proteus vulgaris* (Schnitzler), etc., are also observed.

In the secondary cystites the agent of the primary infection is frequently found,—as, for example, the gonococcus in post-gonorrhœal cystitis, the streptococcus in puerperal cystitis, the tubercle bacillus in cystitis of the tuberculous.[1]

The method for the bacteriological examination of the urine in cystitis is the same as that which we have mentioned in speaking of nephritis.

The urine is obtained by extracting it from the bladder with a sterile catheter; it is immediately centrifugalised,

[1] Barlow, "Aetiologie der Cystitis," 'Arch. f. Derm.,' 1893; Bumm, "Zur Aetiologie der puerperalen Cystitis," 'Centralblatt für Bakt.,' 1886; Huber, "Aetiologie der Cystitis," 'Virchow's Arch.,' Bd. cxxxiv, 1893; Morelle, "Ét. bactériologique sur le cystites," 'La Cellule,' vii, 1891; Müller, "Aetiologie der Cystitis," 'Virchow's Arch.,' cxxix, 1892; Schnitzler, 'Zur Aetiologie der Cystitis,' Wien, 1892; Denys, "Étude sur les infectiones urinaires," 'Bull. de l'Acad. Royale de Méd.,' Bruxelles, 1892; Krogius, 'Recherches bactériologiques sur l'infection urinaire,' Helsingfors, 1892; Schow, "Ueber einen gasbildenden Bacillus im Harn bei Cystitis," 'Centralblatt f. Bacter. und Paras.,' Bd. xii, 1892; Tricomi, "Sulla eziologia della cistite," 'La Riforma medica,' 1889; Bumm, 'Die gonorrhöeschen Schleimhauterkrankungen,' Wiesbaden, 1887; Bizzozero, 'Microscopia clinica,' Milano, 1895.

and then the sediment is examined. With the sediment microscopic preparations are made for the research for the pyogenic microbes, or the *Bacterium coli*, or the gonococcus, or, finally, the tubercle bacillus, making use of the diverse methods indicated for the demonstration of these bacteria.

Cultures are also made in gelatine and agar, especially for studying the pyogenic microbes, the *coli*, and verifying whether mixed infections exist. In the cases in which the suspicion of tuberculous cystitis is not confirmed, or is contradicted by the direct microscopic examination, inoculations of the sediment should be made in guinea-pigs.

As a rule, as Bizzozero has well observed of the bacterial cystites, one can nearly always diagnosticate by simple microscopic examination those depending on the gonococcus and on the tubercle bacillus, two bacterial species easily recognisable by their staining, form, and characteristic disposition.

It is precisely the demonstration of these two species which has great importance for therapy, inasmuch as it indicates the treatment.

In *endometrites*, in *parametrites*, in *salpingites*, the bacteriological examination has acquired great importance, and has entered largely into gynæcological practice.

The etiology of endometrites is certainly very varied, and not yet completely studied. But some very common forms are well known, which present a diverse etiology, and therefore offer different indications for the relative treatment.

No one can fail to recognise the importance for the diagnosis and treatment of finding the tubercle bacilli in the secretion or in the products of the scrapings, or the gonococcus instead of the *Bacterium coli* and the pyogenic staphylococci that are met with in other forms.

As is known in gynæcological practice, partial scrapings of the uterine mucosa are now frequently adopted for diagnostic purposes.

On the material thus obtained with due antiseptic

precautions, the bacteriological examination is made to obtain positive arguments in relation to the clinical diagnosis.

Thus the use of the exploratory puncture has entered into practice to enable us to recognise the nature of parametrites and salpingites.

In puerperal parametrites staphylococci or streptococci, little virulent, are constantly found; the same micro-organisms are also met with in many forms of salpingitis. But in the greater number of the cases of salpingitis and pyosalpingitis modern researches have demonstrated the presence of the gonococci, and consequently the specific and contagious nature of the disease.

From such diagnosis the therapeutic criteria and the indication of prophylactic provisions for preventing the diffusion of the contagion are obtained.

In the discharge of *gonorrhœal urethrites* a micro-organism is constantly found, to which to-day, after many contradictions and doubts, a positive diagnostic value is attributed. This micro-organism is the gonococcus of Neisser, which is met with in all the gonorrhœal exudates. It presents the form of diplococci consisting of elements that are in contact with one another with a flattened surface, so that they have the appearance of a coffee berry. The division, as in many other diplococci, takes place in such a way that each element, dividing into two, is detached from its companion. As the lines of division are perpendicular to one another, it follows that the proliferating cocci never form chains, but clumps.

The characteristic of the gonococcus does not consist in its form nor in its reactions to the staining substances; it consists in its mode of action in respect to the protoplasm of the pus-corpuscles; the gonococcus is always observed within the leucocytes, and forms accumulations, sometimes very plentiful, within the nuclei.

The gonococcus has been found not only in urethral gonorrhœa, but also in blennorrhagic conjunctivitis, in gonorrhœal endometritis, in many forms of salpingitis and of pyosalpingitis, in certain cases of proctitis, and of vulvo-vaginitis in infants.

The recent studies of Wertheim[1] and others have also demonstrated the gonococcus in parametritis, in ovarian abscesses, and those of Bordoni Uffreduzzi in gonorrhœal arthritis.

For the bacteriological diagnosis of blennorrhagia the majority of authors deem the simple microscopic preparations sufficient.

To make the preparation a drop of the gonorrhœal pus is placed on the cover-glass, is distended with a needle, is dried, passed through the flame as usual; then it is stained with a solution of carbol-fuchsin, washed in water, dehydrated, and mounted in balsam.

As a rule these preparations are sufficient for the diagnosis, but in doubtful cases of gonorrhœal arthritis, of salpingitis, etc., cultures should be made.

For making cultures according to Monti,[2] Wertheim,[3] etc., the gonorrhœal pus is sown in tubes of liquid *human serum;* to each tube of serum is added an equal volume of glycerine agar (2 per cent.), liquefied and brought to a temperature of 40° C. These mixtures are then rapidly poured into sterile capsules, where they solidify. The capsules are next placed in the incubator, and after twenty-four hours the development of the gonococci colonies has already taken place. These colonies are greyish white, finely granular, with a more obscure dot in the centre if they be superficial. The deep, on the contrary, are more compact and nodulated.

The microscopic diagnosis is, as we have already said, of great advantage, indeed nowadays it is considered indispensable, especially in doubtful cases.

A few years ago the pathogenic and diagnostic value of the gonococcus was much discussed. Some specialists for a certain time believed that the presence of the gonococcus did not necessarily indicate the specific nature of the disease, but more recent researches have demonstrated, also by experiments in man, that the gonococcus is in reality the

[1] Wertheim, 'Deutsche medizinische Wochen.,' 1890.

[2] Monti, 'Sulla causa della blennorragia,' Soc. Med. di Pavia, 1890.

[3] Wertheim, "Reinzüchtung der Gonokokken durch Plattencultur," 'Deutsche med. Wochen.,' 1891.

cause of blennorrhagia, and the studies made on the microbe enable us to distinguish it from the other micro-organisms which are present in ordinary pus.[1]

In acute blennorrhagia the gonococci are always very plentiful, and it is therefore easy to find them and to verify the diagnosis.

In chronic gonorrhœa the gonococci are found with much difficulty, because their number is very small, and their appearance in the discharge is very irregular. For verifying the diagnosis in such cases it is useful to inject for some days into the urethra a solution of sublimate 1 in 20,000; thus one obtains an irritation of the mucosa, which manifests itself with the production of an abundant secretion, that in its turn causes a desquamation of the urethral epithelium. Then the gonococci deeply nested in the mucosa are brought to the surface, and are met with in the secretion. If after this expedient the cocci are not found in the discharge, one can maintain that the infective period of the gonorrhœa is extinct.

It is superfluous, as Bizzozero wisely writes, to dwell on the importance of the diagnosis which the microscopic demonstration of the gonococci has in some cases. Even confining ourselves to acute urethritis, by means of the gonococci we shall be able to decide between a gonorrhœa and an urethritis from a soft sore, or in the case of an inflammatory phimosis with a purulent discharge one will be able to decide whether the latter is due to a gonorrhœa or to a simple balanitis.[2]

In *soft ulcer* Ducrey [3] and others have demonstrated the constant presence of a very characteristic bacillus, which up till now has never been cultivated. In sections of the little edges, excised from the ulcer, the bacilli, well stainable by Löffler's fluid or by carbol-fuchsin, present such a

1 Bumm, 'Der Mikroorganismus der gonorrhoischen Schleimhauterkrankungen,' Wiesbaden, 1885; Wertheim, "Die ascendirende Gonorrhöe beim Weibe," 'Arch. f. Gynäk.,' Bd. xli, 1891.

2 Bizzozero, 'Microscopica clinica,' Milano, Vallardi, 1895.

3 Ducrey, "Ricerche sperimentali sul virus dell' ulcera molle e dei bubboni," 'Giornale italiano delle malattie venerea,' 1889, No. 4; see also 'Monatshefte für prakt. Dermatologie.'

typical appearance that they cannot be confused with any other known micro-organism. They form longish chains, frequently parallel in the superficial part of the ulcer, especially when there is a notable leucocytic infiltration. In the deeper zones many bacilli are also observed within the cells.

Even in the secretion of the ulcer these bacilli may be found; they are generally within the leucocytes. The most recent studies tend to give a real diagnostic value to the presence of Ducrey's bacillus, which has been constantly met with in soft ulcers by Krefting, Unna, Quinquand, Nicolle, Patersen, Dant, Kruse, and others.

The bacteriological examination has entered also into diverse other specialities, and has furnished notable advantages for the diagnosis of various diseases.

In diseases of the eye it is very useful, especially for the diverse forms of *conjunctivitis* and *keratitis*.

In ordinary and simple conjunctivites, staphylococci, streptococci, and other micro-organisms are found. The research for the pneumococcus has differential importance for the diagnosis between blennorrhagic conjunctivitis and other common and grave forms generally due to the streptococci. Thus the demonstration of the diphtheria bacilli has great diagnostic value in pseudo-membranous conjunctivitis.

It is readily understood how such diagnostic discoveries furnish precious indications for therapy.

The discovery of the tubercle bacilli in certain forms of chronic granular conjunctivitis has also great diagnostic and prognostic importance.

In the *lachrymal canals* and in the lachrymal sac concretions formed by the development of the mycelia appertaining, for the most part, to the *Cladothrix Försteri* have been frequently met with.

Otiatrics also has been able advantageously to apply bacteriological diagnosis in various diseases of the external and of the middle ear.

In the *external auditory meatus* coatings and concretions formed of diverse fungi have been found, such as the *Aspergillus niger*, *A. flavus*, *A. fumigatus*, and *A. nidulans*, the

Eurotium repens, and *E. malignum*, the *Mucor corymbifer* and *M. septatus*, the *Penicillium minimum*, the *Verticillium graphii*.

In the pus of otites mediæ the common pyogenic microbes, pneumococcus, bacillus of Friedländer, *Bacillus pyocyaneus*, have been met with.

In many chronic forms the tubercle bacillus has been found, and the bacillus of Löffler in the diphtheritic forms.

The importance of these discoveries has not escaped the attention of specialists, and in the relative journals a rich literature is already to be found.

In *cold abscesses* the bacteriological examination is negative, as Rosenbach, König, and others have already noted. These abscesses are due to carious processes of the bones determined by the tubercle bacillus. In the puriform liquid extracted the bacilli are not discovered by microscopic examination, because they are very scarce; but if the exudate be inoculated in a guinea-pig this animal dies of tuberculosis, abundantly demonstrating the characteristic bacilli.

In *arthrites* and in hydrarthroses of tubercular origin the finds are equal to those mentioned for cold abscesses.

In arthrites of rheumatic origin, even when accompanied by considerable liquid effusion, no micro-organisms have up to now been met with. In purulent arthrites the ordinary pyogenic microbes, and especially the streptococci, are found.

Of gonorrhœal arthrites we have already spoken when dealing with blennorrhagia.

There are, finally, some forms of arthrites with abundant fibrinous exudate, which arise in the course of pneumonia and of endocarditis, and are characterised by the diplococcus of Fraenkel-Weichselbaum (Weichselbaum, Monti, Netter, Lannelongue, Hachard, etc.). These forms of arthrites, which can be well diagnosticated by means of an exploratory puncture, are much more benign in relation to their etiology than the arthrites caused by the pyogenic microbes.

In dermatology the bacterial examination has offered undoubted advantages for the diagnosis of various diseases.

In *tinea favosa*, Schönlein, in 1889, discovered a special fungus. If we examine a fragment of a crust in water or glycerine, we readily recognise the septiferous and ramified mycelium tubes of the *Achoron Schönleinii* ; in the centre of the crust conidia of a round or oval form are nearly always found, frequently with yellowish contents.

This fungus is met with not only in tinea of the hairy scalp, but also in the favi which develop on the skin deprived of hairs and in the nails. For the diagnosis the clinical characters and the microscopic examination are sufficient; the latter is made, examining fragments of the favus broken up in glycerine, or better in water acidulated with acetic acid.

For the examination of the hairs those little pigmented are preferable, and they are softened in caustic potash. The Achorion invades the sheaths of the root as well as the shaft. The cultivation of the *Achorion Schönleinii* is easy. In gelatine plates develop the white colonies, stellate, rayed, constituted by the mycelia only ; they rapidly liquefy the nutritive medium. In serum at 37° C. the formation of the conidia takes place.

In *herpes tonsurans* the *Tricophyton tonsurans*, which is also the cause of parasitic sycosis, of eczema marginatum, of tricophytic onychomycosis, and of other cutaneous affections is found.

For the microscopic demonstration of the Tricophyton it is sufficient to pluck out the hairs and soften them with potash, scrape the epidermis, and treat the material thus obtained with potash or acetic acid.

The fungus consists of long straight mycelium tubes, little ramified, and thinner than those of the Achorion; the conidia are smaller and less numerous. The latter abound only in the shaft of the hairs, and this is a differential criterion in respect to the Achorion.

The Trichophyton also can be cultivated; its colonies in gelatine resemble those of the Achorion, but they are relatively more robust, and liquefy more rapidly.

The research for this fungus has great importance, because

the morbid forms produced by it are very contagious, but easily curable when recognised. Frequently the clinical characters alone are insufficient for the diagnosis, because they permit the dermatoses caused by the Tricophyton being confused with other forms entirely different.

In *pityriasis versicolor* another fungus, the *Microsporon furfur*, is found. It is constituted of roundish thick conidia from 4 to 6 μ, united into numerous groups, and of thinner filaments, flexuous, which insinuate themselves between the epidermic lamellæ.

The examination and demonstration of this fungus has very little importance; it is important only to know this species in order not to confound it with the preceding.

In *malignant pustule* the bacteriological examination is of capital importance, because it is the sole means by which the diagnosis can be positively established.

We have several times seen cases of suspected pustule in which it was necessary to give an immediate opinion for the treatment, and also for medico-legal requirements, but the clinical criteria were absolutely insufficient. In similar cases the diagnosis can be positively made by means of microscopic preparations and cultures.

From the suspected pustule the exudate is squeezed as much as possible, and the last drops of it are collected, because the first are contaminated with saprophytic germs. A drop of the exudate is distended on a cover-glass and stained by Gram's method (see paragraph on the pyogenic microbes). On examining the preparation, when it treats of anthrax, rather thick bacilli are found, frequently disposed in a series of two or three individuals, each of whose ends instead of being rounded presents a slightly concave surface of contact. When instead of anthrax we have to do with a carbuncle, then in place of the bacilli described innumerable pyogenic cocci are met with. There are also cases of mixed infections, in which the pyogenic microbes are associated with the *Bacillus anthracis*. If we have doubt as to the appearance of the bacilli observed by simple microscopic examination, cultures should at once be

made, which will establish the diagnosis in a definite way.

For making the diagnosis with cultures we believe agar to be useful, in which, at 37° C., after twenty hours, or even less, absolutely characteristic colonies are observed. Some tubes of agar are melted, and are cooled to below 40° C., then with the platinum needle a drop of the exudate, obtained as above described, is placed in the first tube; the tube is gently shaken, then with the platinum loop three small drops of the liquid of this first tube are introduced into a second tube. The contents are rapidly turned into two sterile capsules, are allowed to solidify, and finally are placed in the incubator. After from twelve to twenty hours colonies can be well recognised under the microscope. These colonies present an extraordinarily characteristic development of threads; especially in the superficial ones, where the tufts of threads expand on the agar, beautiful wavy wreaths like locks of hair are observed. It is sufficient to have once seen these colonies to recognise them afterwards at a glance.

By the same method cultures disseminated in gelatine also can be made, where the colonies are visible after forty-eight hours or so as small dots surrounded by a serpentine tail. These colonies, as they gradually become superficial and begin to liquefy the gelatine, become more lax, and present the characteristic Medusa head appearance.

He who desires further confirmation should make inoculations in guinea-pigs. As a rule, however, the microscopic examination and cultures are more than sufficient for a rigorous diagnosis.

The bacteriological examination is of truly exceptional advantage in the diagnosis of anthrax in man, such disease sometimes producing at first, instead of a malignant pustule, large bullæ and vesicles full of serum, which very much resemble the vesicles and bullæ of acute pemphigus.

We have observed a case of this kind where the diagnosis was established only by means of cultures, because the bacilli were very scarce in the serum obtained by puncturing the bullæ.

As is known, there are also cases of intestinal anthrax

from the ingestion of infected meat, and cases of anthrax by inhalation (some forms of diseases of rag-pickers). In these cases the diagnosis can be positively established by means of cultures only.

In intestinal anthrax there are generally vomiting and sanguinolent diarrhœa. In these materials, however, multiple micro-organisms may be found, and an inexpert observer would not easily distinguish the anthrax bacilli among them.

It is therefore important to make disseminated cultures, with diverse attenuations both of the vomit and the fæces.

In anthrax by inhalation there is always an abundant frothy sputum, generally sanguinolent. Here, also, cultures only give an absolute criterion for establishing the diagnosis.

The examination of the blood in cases of anthrax infection is of great advantage for the prognosis. At the beginning of the infection bacilli are never observed in the blood; when they are found there, even in very small quantity, that is, scarcely recognisable by the method of cultures, the disease may be considered absolutely fatal.

Malignant pustule may, as we have stated, be confounded with carbuncle. The bacteriological examination, however, enables us readily to establish the diagnosis, since in carbuncle, as in ordinary boils, pustular acne, etc., the common pyogenic microbes already described are found.

From anthrax it is also necessary to distinguish another infection, in reality very rare and little studied, met with in Italy by Bordoni-Uffreduzzi,[1] Foà, Banti, and others. This disease is determined by a bacillus very similar to that of anthrax, but it differs from it, inasmuch as the bacillus (*Proteus capsulatus*) forms shorter elements with rounded ends, provided with a very evident capsule. The cultures are well distinguished, because they form white colonies, compact, raised, and do not liquefy gelatine.

[1] Bordoni-Uffreduzzi, "Ueber den *Proteus hominus capsulatus*," etc., Zeitschrift für Hygiene,' vol. iii, No. 2.

Another disease that might be confused with anthrax is *malignant œdema*, a very rare form nowadays in human pathology.

This disease has been described by the French surgeons with the name of gaseous gangrene. In 1868 Bottini [1] had an opportunity of studying the disease in Italy, and he demonstrated its infective nature, repeatedly transmitting it to animals. The specific micro-organism was first recognised by Pasteur,[2] and it was described under the name of *vibrion septique*, and isolated in pure culture by Liborius.[3]

The importance of this micro-organism in the pathology of man was demonstrated by Arloing[4] and by Nékàm,[5] who, after having observed two typical cases of malignant œdema in man, and having made the bacteriological diagnosis, collected all the cases of this disease (about fifty) which up to then had been published.

The bacilli of malignant œdema are easily distinguished from those of anthrax, because they are very motile, while those of the latter are non-motile. In the organism they form long filaments, while the anthrax bacilli in the organism present only isolated elements or united in small numbers. In fine, the bacillus of malignant œdema does not stain with Gram's method, while the anthrax bacillus does.

From the point of view of the cultures there is also a capital difference; the bacillus of malignant œdema is anaërobic,—that is to say, it develops only in an atmosphere deprived of oxygen.

Glanders in man is not so rare as is believed, especially among stablemen, coachmen, and others who have to attend to horses. As Strümpel has well observed, the diagnosis of glanders is very difficult when the bacteriological examination is wanting. Furthermore, we can say that up to a few

[1] Bottini, 'Sulla gangrena traumatica invadente,' Milano, 1868.

[2] Pasteur, 'Bulletin de l'Académie de Médecine,' 1877–81.

[3] Liborius, 'Zeitschrift für Hygiene,' vol. i.

[4] Arloing, "Septicémie gangréneuse," 'Leç. sur la Tuberc. et certaines septicem.,' Paris, 1892.

[5] Nékàm, "Az œdema malignumról.," 'Magiar Orvosi Archivum,' 1892.

years ago, in practice glanders was nearly always confused with pyæmic forms.

Nowadays the diagnosis can be positively made from the beginning, precisely with the criteria furnished by microbiology.

The first manifestations of the disease are always of a local nature, and are generally characterised by cutaneous pustules or abscesses; then follow articular tumefactions and exudates.

Given a suspected case, we immediately collect the material of the pustules or of the abscess with the usual bacteriological precautions, and make disseminated cultures in glycerine agar with or without the addition of liquid serum. At the same time, as Strauss[1] advises, we inoculate a part of the material into the peritoneum of a male guinea-pig. When it is glanders the guinea-pig becomes ill in a characteristic manner, and after two or three days presents a tumefaction of the testis, which subsequently suppurates; the characteristic bacilli are found in abundance in the pus.

The glanders bacilli are very small; stain well with Löffler's alkaline solution of methylene blue, especially with heat; they do not stain with Gram's method.

On glycerine-agar plate cultures they form glistening granular colonies, whitish, with distinct margin; these colonies develop well at 37°—38° C. only. Very characteristic are the cultures on potato, where after two days we observe growth of a honey-yellow colour, which later becomes darker, and reaches a mahogany colour.

In horses the diagnosis is now facilitated by means of inoculations of mallein, which is a glycerine extract of the glanders cultures.

After the injection the glandered horses present a strong febrile reaction, a temperature of 40°—42° C., and œdema of the site of inoculation; the reaction begins four hours after the injection.

Leprosy may be easily confused with tuberculosis, above all in the initial cases. We several times have been in

[1] Strauss, 'Revue vétérinaire,' 1889.

doubt as to the diagnosis, especially in cases of primary leprous nodules of the larynx; the diagnosis, however, can be positively made by extracting a small nodule and making the bacteriological examination.

If we treat the material spread out on the cover-glass or microscopic sections with the method indicated for the staining of the tubercle bacilli, we find that they resist the action of the acid and remain well stained, especially if we make a simple staining. But they differ from the tubercle bacilli because they stain very well with Gram's method, and because they present a characteristic appearance. In fact, in the leprous nodules the bacilli are accumulated within the cells, which thus assume a characteristic appearance, and are called *leprous cells.*

Bubonic pest, considering the continuous relations we have with India, may from one moment to another invade Europe. Fortunately in these latter years the means for a reasonable defence against the dreaded importation of the black death has been much developed.

The plague is no longer that enigmatic disease which, as in former centuries, can cause a panic by its unforeseen invasions, all the more dreaded because it was unknown in its essence and causes.

To-day, owing to the studies of Kitasato,[1] Yersin,[2] Avyama,[3] and Zettnow,[4] we know in a precise way the agent of bubonic pest; we can easily recognise the disease even in the first case imported, and consequently make opportune prophylatic provisions; moreover, Yersin has already found a method of specific treatment, of which we shall speak in another paragraph.

Bubonic pest manifests itself with bubo, generally in the

[1] Kitasato, 'Preliminary Notice of the Bacillus of the Bubonic Plague,' Hong-Kong, July, 1894.

[2] Yersin, "La peste bubonique à Hong-Kong," 'Annales de l'Institut Pasteur,' September, 1894.

[3] Avyama, "Mittheilungen über die Pestepidemie im Jahre 1894 in Hong-Kong," 'Centralblatt für Bakteriologie,' vol. xv, Nos. 12, 13.

[4] Zettnow, "Beiträge zur Kenntniss des Bacillus der Bubonpest," 'Zeitschrift für Hygiene und Infektionskrankheiten,' Bd. xxi, 1896.

groins, less frequently in the axillæ, easily accessible to bacteriological examination. In the pus of these buboes the bacilli are very numerous, especially at the commencement of the process; they are small oval rodlets with rounded ends, sometimes surrounded by a capsule.

They stain readily with the ordinary methods; they are decolourised by Gram's method. According to Aoyama, in an advanced period numerous streptococci are found in the buboes together with the bacilli, which are easily distinguished by their appearance, and because they remain stained by Gram's method.

Cultures serve well for confirming the diagnosis. In glycerine-gelatine, and better still in glycerine-agar, the plague bacilli form white, transparent, and iridescent colonies. In gelatine stab cultures they form a cone of a snowy whiteness, terminated by very thin filaments. In alkaline broth they leave the liquid clear, forming a granular deposit at the sides and foot of the tube.

Inoculated in animals, especially in rats, which are very sensitive, and die in a time varying from one to three days, they produce local œdema, tumefaction of the lymphatic glands, and hæmorrhages of the abdominal parietes. The bacilli are found in all the organs, and even in the blood.

Considering the knowledge we now possess on the prophylaxis of infective diseases, it is not necessary for us to dwell upon the advantages that are to be derived by being able precisely to recognise a first case of imported plague.

Relapsing fever is a disease which up till now has never been prevalent in Italy. The diagnosis of this disease may easily be made by means of the bacterioscopic examination of the blood during the access, as was first demonstrated by Obermeier in 1873. If a preparation of the fresh blood be made during the febrile period, motile spirilla are observed within the red corpuscles.

This micro-organism, which is now called the *Spirillum Obermeieri*, may be even better demonstrated in the blood, making stained preparations with the Günther-Friedländer method already described when speaking of pneumonia. In the apyretic intervals the spirilla disappear from the

blood; they are not found in the secretions or the excretions.

The demonstration of these spirilla is the most positive proceeding for establishing the diagnosis.

Although the bacteriological diagnosis of *typhoid fever* has been considered simple and easy by many authors, still it is surrounded by so many difficulties that it has not truly entered into practice. It is not the case here of thinking of the direct microscopic examination of the blood or of the fæces.

If assistance be required, it must be sought in the cultures or in other technical proceedings which we shall describe.

From the numerous observations which are found scattered in literature, it results that in about half the cases the typhoid bacilli have been cultivated from the urine of the patients. On the other hand, it is very difficult to cultivate Eberth's bacillus from the blood of the rose spots.

Some authors, including Parietti,[1] Philipowicz, Seitz, Grawitz, Karlinski, and others, employing special proceedings, have succeeded in cultivating the specific bacillus from the fæces. But the find is very far from being constant, and the difficulties which are met with in the differential diagnosis, and in the separation of the typhoid bacillus from the *Bacterium coli*, greatly lessen the value of this research, also because it requires a very long time.

Nevertheless, to be complete, we shall here indicate the principal differential notes between the typhoid bacillus and the *Bacterium coli*.

The gelatine plate cultures of the typhoid bacillus develop milky-white non-liquefying colonies very similar to those of the *Bacterium coli*.

But in stab cultures in gelatine or in agar, especially if they contain glucose, the *Bacterium coli* develops gas, while this is never observed in the typhoid cultures. On potato the typhoid bacillus forms an invisible pellicle, while the *Bacterium coli* forms a raised brownish-yellow growth.

[1] Parietti, 'Rivista d' Igiene e sanità publica,' 1890.

Finally the *Bacterium coli* coagulates milk, while the *Bacterium typhosus* develops without producing coagulation. The latter does not give the indol reaction, while the former gives it very distinctly.

But since there are many species of pseudo-typhoid bacilli, and many varieties of *Bacterium coli* which present some of the properties of the typhoid bacillus, so all the above-mentioned proofs are necessary for forming a bacteriological judgment sufficiently founded, which requires an exorbitant amount of time, and cannot therefore be adapted to clinical requirements.

Puncture of the spleen and the successive cultures of the blood extracted have also in many cases been used for the diagnosis, but very frequently this method is not practicable. Very often it does not give the results sought, inasmuch as the cultures remain sterile; for this reason also this method has not great diagnostic value. We have succeeded several times in obtaining pure cultures of the typhoid bacillus by puncturing the spleen; but in most cases we have had negative results. Admitting this probability, we consider it our duty not to advise this painful and little practical method of punctures of the spleen.

A truly practical method seems to have been found lately,[1] owing to the studies of Widal, Pfeiffer and Kolle,[2] Gruber and Durham,[3] and Pfuhl.[4] This last author has verified, especially on the basis of numerous personal experiments, the diagnostic value of the Widal-Pfeiffer method, and has simplified it in such a way as to render it easy for the greater number of practitioners.

Pfuhl has simplified the *technique* in the following mode:

A drop of blood obtained by puncturing the lobe of the ear of a patient suspected of typhoid is diluted with ten

[1] On the diagnostic value of the new method compare also Custani, "Sulla sierodiagnostica del Tifo," 'Gazzetta medica Cremonese,' 1897, No. 2.

[2] Pfeiffer and Kolle, 'Deutsche mediz. Wochenschrift,' 1896, No. 13.

[3] Gruber and Durham, 'Münchener mediz. Wochen.,' 1896, No. 13.

[4] Pfuhl, "Eine Vereinfachung des Verfahrens zur Serodiagnostik des Typhus," 'Centralblatt für Bakteriologie,' 1897, No. 2.

drops of water. With the platinum loop a drop of this dilution is placed in a watch-glass and mixed with a drop of broth culture of the typhoid bacillus. If the patient be really suffering from typhoid the specific reaction quickly occurs; that is, the bacilli are grouped in semi-motile masses, which, however, after five or ten minutes lose their motility. If, on the contrary, the patient has not typhoid, the bacilli remain uniformly diffused and motile in the mixture, and agglutination does not take place.

If the drop of blood obtained is left to dry in the air, and the test is made the following day, dissolving the blood in ten drops of water, the reaction equally takes place. This is, perhaps, the most interesting part of Pfuhl's method, which renders it truly practical, because those practitioners who are not able to perform the test themselves can collect the blood and send it to a specialist for diagnostic judgment.

The bacteriological diagnosis of *cholera* has now entered into universal practice, and has been of great advantage in enabling us to recognise the first cases of the disease, and to adopt those energetic measures of disinfection and isolation that are very frequently so useful in arresting and destroying the contagion.

For the bacteriological diagnosis of cholera it is necessary to proceed to the microscopic examination of the dejections and to the relative cultures. The simple microscopic examination is not sufficient for the diagnosis.

We begin with the microscopic examination, searching for the mucous flocculi (which consist of swollen epithelia) in the characteristic rice-water stools, and spreading them out on the cover-glass, where they are stained with methylene blue.

There are cases of cholera in which such preparations appear almost as pure cultures of the cholera bacillus. Furthermore, in 50 per cent. of the cases, Pfeiffer says, this microscopic examination is sufficient for a trained observer to establish the diagnosis. It is very useful to examine also the soiled linen of the patient, on which, as Koch first demonstrated, the cholera vibrios multiply very rapidly, and

for a time survive all the intestinal bacteria. Nevertheless we must not expect that the diagnosis of cholera is so easy in all cases. Frequently, on the contrary, the cholera bacilli are so mixed with a large mass of other bacteria that it is difficult to recognise them by direct examination.

As a rule, we must have recourse to the cultivation methods to verify the diagnosis. The cultures may be made in two modes :

(*a*) Ordinary disseminated plate cultures; (*b*) peptone cultures with Schottelius' method.

(*a*) To make the ordinary plate cultures a mucous flocculus is taken up with the platinum loop and placed in a tube of melted gelatine; after having mixed it thoroughly with the needle a first attenuation is made, carrying three drops of gelatine from the first tube into a second; then a second attenuation is made, transporting three drops from the second to a third. The contents of the three tubes are poured into three Petrie's capsules, where the gelatine solidifies.

The capsules are kept at a temperature of 20° to 22° C.

After twenty-four, thirty, or forty-eight hours colonies are developed in the cultures, with irregular margin formed of vitreous granules surrounded by a liquefying halo. These colonies consist of the cholera bacilli, which are recognised by microscopic examination. Since in the intestine other vibrios forming colonies of such a characteristic appearance are not found, consequently this find already confirms the diagnosis. When we are still in doubt, stab cultures in broth or gelatine should be made. In gelatine stab cultures the cholera microbe forms a cone of characteristic liquefaction; in peptone broth, or in simple alkaline solution of peptone, after six hours the cholera already presents the indol reaction with the simple addition of a drop of sulphuric acid.

(*b*) There are always cases in which the cholera bacilli are very scarce in the fæces, while other bacteria abound in them; this is verified especially in the fæces emitted for some time and transported to a distance for examination during hot weather. In such cases Schottelius' method for increasing the cholera vibrios, recently much recommended by Koch and by Dunbar, is very useful. To apply this method,

tubes containing a solution of peptone 1 per cent., with ½ per cent. of sodium chloride, are prepared. With the platinum loop a series of these tubes are sown with flocculi of the mucus of the suspected fæces. These cultures are then placed in the incubator, and after six to twelve hours are examined, taking a drop of the material from the surface, which is examined as a hanging drop and in dry preparations. As a rule, a thin pellicle, constituted entirely of the cholera bacilli, is formed after six hours on the surface of the liquid; these bacilli are recognised by making the hanging-drop cultures or the dry preparations. If any doubts should remain, gelatine plate cultures may be made with this material, where the cholera colonies appear with the characteristic notes already described. The intermediary of the peptone culture has the great advantage of multiplying enormously the cholera vibrios, so that they can be easily isolated by means of the gelatine plate cultures.

To verify the diagnosis further, besides the indol reaction obtained by the simple addition of an acid, according to Pfeiffer, inoculations of immunised animals are useful.[1]

These methods, as is readily understood, have the great advantage of permitting the early and positive diagnosis of the first cases of cholera. In these latter years they have been largely employed also in Italy, and have many times been the means of arresting epidemics.

In the *dysentery* of tropical countries amœbæ are met with very regularly in the fæces, especially in the dysenteric mucus. In the mucous flocculi of the acute stages of dysentery the amœbæ are found in innumerable crowds. They are more rare in subacute and chronic dysentery. We must say, however, that amœbæ are also present in variable numbers in the fæces of healthy persons, and consequently their diagnostic value will remain null until we have differential criteria for distinguishing the dysenteric amœbæ.

[1] Pfeiffer, "Die Differentialdiagnose der Vibrionen der Cholera Asiatica, mit Hülfe der Immunisirung," 'Zeitschr. f. Hyg. und Infekt.,' Bd. xix, 1895.

Up till now we have only experimental researches, which tend to demonstrate that the amœbæ of the intestine of healthy individuals (*Amœba intestini vulgaris*) are not pathogenic for animals, while the *Amœba dysenteriæ* found in pure culture in some cases of hepatic abscess (see the relative article) has produced a dysenteric disease in cats.

Only to be complete do we here indicate the method for finding the amœbæ in the fæces. The fæces must be examined in the fresh state, immediately after evacuation. The mucous flocculi are sought for and dissolved in a ·75 per cent. solution of sodium chloride, heated to a temperature of 35° to 40° C. The preparation is then covered with the cover-glass and is examined at once. The amœbæ die very quickly in the fæces, and disappear a few hours after evacuation. In the fresh materials examined on the hot stage the amœbæ still present distinct movements. Conservable preparations are obtained by distending a flocculus of the amœbiferous mucus on the cover-glass, then immersing it in saturated sublimate or in absolute alcohol. From the sublimate the cover-glasses are passed into alcohol, then they are stained with methylene blue or with carmine.

At any rate, seeing the contradictory observations of some authors, who consider dysentery to be produced by bacterial poisons,[1] and the absence of precise characters for recognising the dysenteric amœbæ, it is clear that these amœbæ have not, at least at present, any clinical value.

The discovery of the malarial parasites has been of great advantage for the diagnosis of *malaria*. Before this remarkable discovery the continued fevers were frequently confused with typhoid and other gastric infections: the pernicious fevers have been often confused with intoxications, and also with other very acute infections; the mild quotidians, in special cases, have left the practitioner in doubt between malaria and incipient tuberculosis. The demonstration of the typical malarial parasites in the circulating blood establishes positively the diagnosis, and consequently

[1] Celli, 'Annali dell' Istit. d' Igiene sperimentale di Roma,' 1896, No. 2.

furnishes the therapeutic indications which can save the life of the patient.

For the examination of the blood in malarial infection the best method is also the simplest—that is to say, it is that by which fresh preparations are made without the employment of reagents. A small drop of blood, obtained by puncturing the fleshy part of a finger or the lobule of an ear with a pin, placed on a cover-glass thoroughly cleansed with acid, alcohol, and ether, is as quickly as possible placed on a slide. Immediately, with the fleshy part of a finger covered with a clean piece of linen, or even with the nail of the little finger, a moderate pressure is made on the cover-glass in order to obtain the uniform distribution of the stratum of blood and to prevent the formation of rolls, making the corpuscles present their flat surface; rapidity of manipulation is also required to prevent the shrivelling of the corpuscle.

The malarial parasites, owing to the innumerable studies made within the last fifteen years principally by Italian authors, have been classified among the Protozoa, and precisely amongst the amœbæ.[1] This demonstration was primarily given by Golgi, who was the first to discover the laws of development of the malarial amœbæ, to distinguish them into diverse species, and put them in relation with the clinical forms so as to be able to furnish precise criteria not only for the diagnosis and the prognosis in general, but also concerning the mode of development of the morbid process, the type of the form by which patients are attacked, the diverse phases of the course, the interval between the accesses, the preparation of other accesses, etc.

The possibility of such great precision of data is included in the following fundamental law discovered and developed by Golgi:

"The malarial parasites, like living organisms, complete

[1] For the history of the development of our knowledge on the etiology of malaria, and on the clinical value of the examination of the blood of the same, the following books may be consulted with advantage:—Mannaberg, 'Die Malaria-Parasiten,' Wien, 1893; Thayer and Hewetson, 'The Malarial Fevers of Baltimore,' Baltimore, 1895; Laveran, 'Paludisme,' Paris, Masson, 1891; Monti, 'Sull' Infezione malarica,' Firenze, 1896.

their evolutionary cycle in a well-determined period; this period corresponds to the time included between the onset of two proximate febrile accesses, naturally including the corresponding apyrexia; the onset of the accesses always coincides with the reproductive phase by segmentation (so-called sporulation) of a parasitic generation. Consequently each fundamental type of fever is in relation with a parasite having a definite evolutionary cycle."

Not only from the pathogenic point of view, but also from that of the diagnosis and of the prognosis, malarial fevers are distinguished into two great groups.[1] To the first group appertain the classic intermittent fevers, that is the common tertian and quartan, with their combinations of double quartan and double tertian and triple quartan; it is to these combinations that many quotidians can be referred (bitertian-quotidian or double tertian, triquartan-quotidian or triple quartan). To the second group appertain the fevers which during the hottest season generally prevail in the countries where malaria has greatest intensity and virulence (Roman Campagna, Pontine Marshes, Tuscan Maremme, some regions of Puglie, Basilcata, Sicily, Sardinia, Algiers, Caucasus, India, Southern States of America, Mexico, Central America, Brazil, etc.).

It is understood that the localisation here mentioned is not rigorous, since, even in countries where malaria is mild, it is not excluded that in special circumstances forms referable to this group may arise. It is also for this that the distinction founded on the pathogenic criterion, which naturally is the expression of the difference in the biology and in the prevailing seat of the development of the parasite, is more important than every distinction obtained from the topographical criterion.

Although on this point the studies are not yet quite complete, still it is now proved that in the first group the parasite chiefly exists and in great part completes the phases of its cycle in the circulating blood; in the second group, on the contrary, the parasite chiefly exists and in great part completes its cycle in the internal organs (particularly the

[1] Golgi, "Sulle febbri malariche estivo-autumnali di Roma," 'Lettera a Guido Baccelli,' Pavia, Bizzoni, 1893.

spleen, medulla of the bones, etc.). Besides, it is necessary to remember that a certain degree of localisation always exists even in the first group.

With regard to the diagnosis, it is well to bear in mind that, while in the first group the microscopic examination of the peripheral blood always furnishes positive results in every period of the clinical course, with the characteristic modifications of the parasites, connected with the phases of development which in great part takes place in the blood, in the second group (severe fevers), on the contrary, the specific find in the blood is not absolutely constant, nor do the parasitic forms, which are seen in the circulating blood, present that succession of phases which is so characteristic of the first group; above all, there is no proportionate relation between the hæmatological find and the general manifestations of the malarial infection.

(*a*) *First group.—Intermittent fevers in which the parasite chiefly exists and in great part completes its cycle in the circulating blood* (*classic quartan and tertian with their combinations*).

The malarial parasite presents itself in the circulating blood under the form of amœbæ contained within the red corpuscles, where they develop till the complete destruction of the corpuscle; then they segment, and the access arises in correspondence with the segmentation.

If we examine the blood of an individual affected by a form of classic intermittent a few hours after the termination of an access, we find that among the many perfectly normal red corpuscles there exist others, either scarce or abundant according to the intensity of the infection, containing internally a protoplasmic body which occupies a fifth of the red corpuscle, sometimes without pigment, more frequently with some brown or black granules, non-motile or with amœboid movements.

It is a young parasitic generation which has invaded the red corpuscles, and is developing at the expense of the corpuscular substance. If we make examinations at successive intervals, we find that the amœboid parasites grow more or less rapidly, according to whether they belong to the tertian or the quartan. With the growth of the parasite the

pigment granules contained in the red corpuscles become numerous in proportion to the increased destruction of the hæmoglobin. A few hours before the access the parasites have destroyed the corpuscular substance, and appear in the blood as protoplasmic bodies spotted with pigment granules.

By degrees the pigment collects at the centre in a very distinct clump; contemporaneously in the body of the parasite radial lines appear, which indicate its division into regular segments. The reproduction of the parasite has thus begun, which terminates with the formation of a series of round spores, representing the individuals of the new generation, destined to renew the cycle, invading new red corpuscles. The onset of the febrile attack coincides with the formation of these globular spores.

Such regular succession of phenomena is verified when a single parasitic generation only exists in the blood; but it is necessary to state that two or three generations or parasitic colonies in various stages of development, and maturing at diverse periods of time, may exist contemporaneously in the blood. To each of these generations a febrile access corresponds, and we thus have the known forms of double tertian, double quartan, triple, etc. When the parasitic colonies succeed one another at irregular intervals more or less irregular fevers are observed.

On the basis of these fundamental principles Golgi has developed the concept that the classification of the intermittent fevers and the diagnostic and prognostic judgment must not be exclusively deduced from the clinical data, but also from the etiological criteria.

Furthermore, Golgi has demonstrated that without such criteria in not a few cases it is impossible to refer the fever to one or another type (which may be more benign or more severe). For example, we could not decide whether a quotidian form is a triple quartan or a double tertian, or a quotidian determined by the parasites of the severe fevers. The fevers of the first group or the classic intermittent are distinguished by Golgi into two categories:

(i) Fevers connected with the cycle of the parasites which develop in three days—*Amœba febris quartanæ.*

This sub-group includes the clinical types of the quartan, double quartan, some quotidian (triquartanary or triple quartan).

While the simple quartan is connected with a single parasitic generation, which completes its regular cycle in three days, in double quartan there are two generations, that reach maturity with an interval of one day; the triple quartan is connected with the development of three generations or parasitic colonies, which also follow with a day's interval.

(ii) Intermittent fevers connected with an evolutionary cycle of parasites that develop in two days—*Amœba febris tertianæ.*

To this sub-group appertain the types of the tertian and of some quotidian (bitertianary or double tertian). These are caused by two parasitic generations which develop with a day's interval.

For distinguishing the amœbæ of the quartan from those of the tertian, according to Golgi,[1] the following biological and morphological characters are useful.

Biological Characters.

(1) Differences in the evolutionary cycle: the parasite of the tertian completes its cycle in two days, that of the quartan in three.

(2) Differences in the character of the amœboid movements: the intra-corpuscular amœbæ of the tertian have much more active movements than those of the quartan.

(3) Differences in the mode of acting of the parasite in respect to the substance of the corpuscle which contains it: the parasite of the tertian discolours the corpuscle in a very energetic and rapid manner. While in the quartan the

[1] Golgi, "Sull' infezione malarica," Accad. di Med. di Torino, Novembre, 1885, and 'Arch. delle Scienze med.,' 1886; idem, "Ancora sull' infezione malarica," Società med. di Pavia, 1886, and 'Gazzetta degli Ospedali,' No. 53, 1886; idem, "Sullo sviluppo dei parassiti della febbre terzana e la quartana," 'Arch. per le scienze mediche,' p. 173, 1889; idem, "Dimostrazione fotografica dello sviluppo dei parassiti della malaria," Accad. Med. di Torino, 1890.

corpuscles invaded by the parasites preserve their characteristic greenish-red colour to the last phase of destruction, in the tertian, on the contrary, the parasites expand their decolourising action even into the most distant parts. Consequently it happens that from the first phases of development, when they occupy only a little part of the corpuscles, the latter are pale and discoloured, which condition contributes to making them easily distinguishable from the others. This rapid discoloration of the whole corpuscle is most probably in relation with the rapidity with which the parasite extends its protoplasmic processes into all parts of the corpuscle to its extreme periphery.

(4) In the quartan the invaded corpuscles have a tendency to shrivel; in the tertian, on the other hand, the corpuscles containing parasites usually present themselves as expanded discs, sometimes larger than the normal corpuscles and hydropic.

Morphological Characters.

(1) In the tertian the protoplasm of the parasite has a thinner and more delicate appearance, and has more distinct contours than that of the quartan.

(2) Relative differences of the pigment: in the parasite of the quartan the pigment presents the form of granulations or thicker rodlets of a darker colour. In the tertian the pigment appears in the form of very fine needles having a brownish colour. Besides, owing to the active amœboid movements of the parasite, the pigment frequently changes its position.

(3) Differences in the mode of segmentation: they are very evident, and such that by themselves alone they permit of the differential diagnosis between the tertian and the quartan. The parasites of the quartan in general are segmented into eight to twelve daughter-spores (daisy formation), much larger than those of the tertian; internally, especially during the division, they show a nucleus visible even in the fresh preparation. The parasites of the tertian multiply, segmenting into a much larger number of daughter-

spores (from twenty to forty); sometimes the segmentation commences at the periphery, and it then has a sunflower formation.

(*b*) *Second group.—Intermittent fevers whose pathogenesis is connected with parasites which chiefly exist and in great part complete their cycle in the internal organs.*

To this group appertain those fevers that dominate in the countries of intense malaria, and assume various types: not rarely they assume with a certain regularity the quotidian type; more often they are very irregular. Frequently they present themselves with the type of continued or subcontinued fevers, and even of the pernicious. They contain febrile types, in which the very prolonged access includes a part of two days (*malignant tertian* of Marchiafava, *bidue* of Baccelli).

In the circulating blood the parasite is represented by small intra-corpuscular non-pigmented amœbæ, sometimes non-motile and in the form of rings or small discs, at other times with active amœboid movements (so-called *plasmodia* of Marchiafava and Celli). With regard to this discovery, from the point of view of the clinical criteria for the diagnosis of malaria and its relative course, it is necessary to note that the data are very far from including the precision and the possibility of being referred to well-determined laws, as happens for the classic tertian and quartan, as Baccelli[1] and Golgi have persistently stated.

In this group there is no proportional relation between the find in the circulating blood and the intensity of the general manifestations, it having been verified that the severest febrile attacks may be in relation with extremely scarce finds; nor with regard to the hæmatological find can we verify the typical modifications connected with the successive phases of development to reproduction, which, on the contrary, are so easily observed in the classic tertian and quartan fevers. In this second group, also, the parasite completes a cycle, but the phases of this cycle do not develop in the circulating blood, but in the internal organs, in which the parasite has a relatively fixed seat (see Golgi—Letter to Baccelli).

[1] Baccelli, "La malaria," 'Policlinico,' May 15th, 1896.

Of the young forms, resulting from this reproduction in certain organs, a great or less quantity—with differences connected with circumstances not yet well determined—may eventually enter the circulation, where it presents the known characters of the small intra-corpuscular amœbæ.

It is certain that the cases with very scarce finds are somewhat frequent in the severe forms. From this it necessarily arises that when one has reason to doubt that he has to deal with this form of malarial infection he should examine the blood with the greatest care and persistence. If we consider also that in these cases the clinical manifestations may simulate other infectious forms, it is readily understood that an accurate and persistent examination of the blood has even greater importance in these cases than in the malarial infections of the first group.

In these cases, doubtful for the diagnosis, certain as to the malarial infection, the verification of very few small intra-corpuscular forms will be sufficient.

The parasitic forms which are found in the circulating blood during the course of these severe fevers are the following:

Small amœbæ, without pigment, sometimes in the form of a disc or a circle, within the red corpuscles, generally well preserved.

Amœbæ a little larger, with few pigment granules, frequently contained in necrotic or shrivelled corpuscles (the brassy corpuscles of Marchiafava and Celli).

Amœbæ a little larger than the preceding, occupying only a fifth of the red corpuscle, containing a small clump of central pigment.

These are the most common forms during the first days of severe primary malarial infection. The segmentation forms are absolutely exceptional in the circulating blood; they are readily found, on the contrary, by puncturing the spleen. This is a confirmation of Golgi's law on the development of the parasites of these fevers in the internal organs.

In the severe recurrent fevers, or in a second period of the severe fevers, the so-called crescent forms, which have a sickle shape, the length of a red corpuscle, containing a clump of pigment granules, are met with in the circulating

blood. Certain round or oval bodies, pigmented, provided with a double contour, also appertain to the cycle of development of these crescent bodies. These parasites, according to Canalis[1] and others, represent a second cycle of development of the parasites of the severe fevers, a slow cycle that is completed in a variable period of five to fifteen days.

As Golgi[2] has demonstrated, certain intermittent fevers with long intervals, already well known to the old clinicians, are in relation with these parasitic forms.

For the clinical microscopic diagnosis of these cases it is well to remember the following facts:

(1) In the course of a series of days of apyrexia the so-called very characteristic crescent forms alone are observed in the blood.

(2) During the accesses the crescent forms disappear, and the small intra-corpuscular amœbæ (young forms) then appear in variable numbers, to disappear anew.

(3) Frequently in relation with the crescents the flagellated forms are found, which, however, also may appear during the development of other parasitic forms,—the tertian for example.

(4) The appearance of the flagellated forms, which Grassi[3] considered the phenomena of death of the parasite, has been up till now observed in proximity to the febrile accesses.

The diagnostic criteria positively derived from these admirable etiological studies have been of immense advantage for the clinic. If we read any treatise on special pathology of only a few years ago, we shall see it stated that the diagnosis of malaria in many cases is extremely difficult.[4] We read that malaria may be confounded with phlebitis, cryptogenetic pyæmia, endocarditis, and even with early

[1] Canalis, 'Sulla varietà parassitaria delle forme parassitaire del Laveran, e sulle febbri malariche che ne dipendono,' Roma, 1889; idem, "Sulla infezione malarica," 'Arch. per le Scienze mediche,' 1890.

[2] Golgi, "Sulle febbri intermittenti malariche a lunghi ed inegolari intervalli," Società medica di Pavia, Maggio, 1889, and 'Archivio per le Scienze mediche,' 1890.

[3] Grassi e Feletti, "Contribuzione allo studio dei parassiti malarici," 'Accademia Giœnia di Catania,' 1892, vol. v.

[4] Strümpell, 'Specielle Pathologie,' 2nd ed., 1885.

tuberculosis; that the pernicious fevers may be mistaken for intoxications, and so on.

To-day all these doubts have no reason for their existence; the research for the malarial parasites and their demonstration enable the diagnosis to be established with the greatest certainty and in a very short time.

The recent studies of English, American, Italian, and German investigators have thrown new light on the paths of transmission of infective diseases, demonstrating in an undeniable way the part that certain species of *Arthropoda* take as intermediate hosts of certain pathogenic micro-organisms. It was the discovery of the relations between mosquitoes and malaria which, owing to its great importance in respect to human pathology, has especially attracted the attention of the scientific world.

The theory which considers certain insects or *Arachnida* as the carriers of miasms and contagions was common even in olden times in various parts of the world. Nuttall[1] in his works has diligently collected an almost complete history of the old hypotheses and of the recent observations in relation to this much-debated question, which has at length been definitely solved.

I shall record only that in many parts of Italy, and in certain regions of Africa and America, it has been commonly believed that mosquitoes are the carriers of malaria.

Angelo Alessandrini,[2] in his article on "Rome and Latium from the Agricultural and Hygienic Points of View," says that "the mosquito with the vehicle of the unhealthy air passes from the Campagna to inhabited places, invades habitations, hides itself by day to hum during the night in search of man in repose, . . . and by means of its bite inoculates him with its poison."

[1] Nuttall, "Die Rolle der Insekten Arachniden (Ixodes) und Myriapoden als Träger bei Verbreitung von durth Bakterien, und thierische Parasiten verursachten Krankheiten des Menschen und der Thiere," 'Hygienische Rundschau,' 1899; "Die Mosquito-Malaria Theorie," 'Centralb. f. Bakter,.' vols. xxv—xxvii.

[2] Alessandrini, "Roma ed il Lazio del punto di vista agrario ed igienico," Annali di agricoltura,' 1871.

Stanley, in his book 'In Darkest Africa,' states that the celebrated explorer Emin Pasha was convinced that a good mosquito net was the best means of preventing marsh fevers. Captain Casati on his return from Africa told me that he fully agreed with this opinion. After Manson[1] had discovered that the filaria of human blood has a mosquito for its intermediate host, the hypothesis of the relations between suctorial *Diptera* and malaria seemed to be scientifically very probable, and as such it was accepted by Laveran in 1890, and by Koch in 1892. But it was Smith and Kilborne,[2] two American observers, who disclosed the triumphant way to the new discoveries by demonstrating that Texas fever, a disease of oxen produced by the *Pirosoma bigeminum*, a parasite very similar to the malarial amœbæ of man, is transmitted by the bite of a special tick (*Rhipicephalus annulatus*), which from the small size of a pin's head attaching itself to an ox and sucking its blood becomes very big, and then falls off and ovulates. The daughter-ticks developed from the ova are capable of transmitting the disease, infecting other oxen with their bites. It has also been experimentally proved that the *Rhipicephalus* is the intermediate host of the *pirosoma*, and that it is found in all the countries where bovine malaria prevails. In Italy this fact was confirmed by Grassi, who, studying bovine malaria in the Agro Romano, discovered the presence of the *Rhipicephalus*, which up to then was believed not to exist in Italy.

Guided by these facts, Manson,[3] who had previously shown the mosquito to be the intermediate host of the filaria, studying the parasites of human malaria, thought that the flagellated bodies, which do not appear in the blood till after it is extracted from the vessels (they have therefore

[1] Manson, "On the Development of *Filaria sanguinis hominis*, and on the Mosquito considered as a Nurse;" a paper communicated by Cobbold to the Linnean Society, March 7th, 1878.

[2] Smith and Kilborne, 'Investigations into the Nature, Causation, and Prevention of Texas or Southern Cattle Fever,' Bull. No. 1, Bureau of Animal Industry, U.S. Dept. of Agricult., Washington, 1893.

[3] Manson, "On the Life History of the Malaria Germ outside the Human Body," 'Lancet,' 1896, vol. i, p. 517, etc.

been considered as degenerative forms by many authorities), were the propagating agents of the parasite in some suctorial insect such as the mosquito, flea, etc.

Ross,[1] impressed by Manson's idea, devoted himself to the study of the question in India, and after many attempts which led to no definite results in relation to human malaria he succeeded in demonstrating that the hæmatozoa of birds have another life cycle in the body of grey mosquitoes, which thus represent the means for the diffusion of malaria. In the blood of birds (*Passer indicus*, *Ploceus bengalensis*, etc.) the hæmosporidia of the genus *Proteosoma*, Labbé, develop at the expense of the red corpuscle, and reach a sexual multiplication, or give rise to the production of free forms in the plasma, the smaller of which (microgametocytes) emit flagella or spermoids; the larger forms do not emit flagella, and are called macrogametes or ovoids. In the blood extracted from birds one may see, according to MacCallum's observations, the spermoid fecundate the ovoid. After this fecundation the second life cycle of the hæmosporidia begins, which is completed in the body of the mosquito. Ross made mosquitoes bite infected birds, and systematically dissecting a mosquito each day he discovered the cycle of the parasite in the body of the mosquito. On the first day there are only the free forms, many of which have protruding flagella. It is very probable that if fecundation has not already taken place in the bird's body it occurs now between the spermoids and the ovoids in the middle intestine of the mosquito. On the second day the pigmented bodies are seen attached to the external coat of the stomach, immobile, with a spheroidal contour; they then increase in size, protrude into the body-cavity, and finally reach a diameter of 60 μ, so that they are almost observable with the naked eye. This evolution is completed in a time which varies according to the temperature, that is from six days in the warm season to a fortnight in the cold season. Having reached maturation the parasites present themselves as cysts full of filaments or sporozoites, which Ross has called germinal rods, among which are found some

[1] Ross, "Du rôle des moustiques dans le paludisme," 'Annales Pasteur,' No. 2, 1899; see also Manson, 'British Medical Journal,' June 18th, 1898.

brown bodies that Ross calls black spores, and considered them to be resisting forms capable of living in water and of continuing the propagation of the parasite from one generation of mosquitoes to another. According to Grassi, Bignami and Bastianelli, the brown spores are, on the contrary, involution forms which lead to the death of the parasite. Be this as it may, after complete maturation the cyst bursts and the sporozoites discharge themselves into the body-cavity; from thence they reach the salivary glands. From the secreting cells of these glands the germinal rods pass with the saliva into the bite made by a mosquito in the skin of a healthy bird, and there mixing with the blood produce the infection.

Ross by numerous experiments has shown that infected mosquitoes communicate the Proteosoma to healthy birds; he thus arrived at the discovery, confirmed by Koch, Grassi, Celli, etc., of the transmission of malaria to birds by the agency of mosquitoes. Koch [1] has since studied more fully the development and diffusion of the *Proteosoma,* Grassi, in the *Culex nemorosus.* While Ross was completing these splendid researches on malaria of birds, Grassi devoted himself to solving the problem of human malaria from another standpoint, beginning by determining which are the species of mosquitoes that are constantly found in malarious places. After many epidemiological researches he, in September, 1898, succeeded in indicating the *Anopheles claviger* as the carrier of human malaria; and Bignami, for the first time, succeeded in communicating malaria to a man, making *Anopheles,* captured in a house where an infected person was living, bite him. Soon afterwards Grassi, with the valuable co-operation of Bignami, Bastianelli, and Dionisi, demonstrated the life cycle of the æstivo-autumnal parasites in the body of the *Anopheles.*

Man, according to Grassi, is the temporary host of the malarial parasite, while the mosquito is its definitive host. In man the malarial amœbæ develop within the red corpuscles, and having reached maturation they reproduce

[1] Koch, "Ueber die Entwicklung der Malariaparasiten," 'Zeitschrift f. Hygiene,' vol. xxxii, 1899.

themselves without fecundation, dividing into many roundish little germs (sporozoites), each of which in its turn invades a red corpuscle, where it grows and multiplies in a similar manner.

Owing to the studies of Smith and Kilborne, MacCullum, Ross, Grassi, and Koch, we now know that all the species similar to the malarial parasite are obliged from time to time to intercalate between the generations without sexual fecundation like that above mentioned, a generation preceded by fecundation with distinction of sex.

Neither fecundation nor sexual generation can take place in the human body. This fact is a considerable check to the very great reproduction of the malarial parasite. However, in the red corpuscle of man individuals of the male and female sexes are found, individuals which remain virgins, never coming into contact with each other. These individuals, already known by the name of free pigmented or crescent bodies, are called gametes in general, and more precisely the females are called ovoids and the males spermoids. Inasmuch as the ovoid is much larger than the spermoid it is also called *macrogamete*, and the spermoid *microgamete*. The spermoids are never found free in human blood, but bodies are met with in it which are capable of instantaneously emitting several of them; these bodies are called microgametogens. They do not emit spermoids in the circulating blood; thus is explained the reason why fecundation of the parasites does not take place in the blood of man. Grassi had observed for a long time that the formation of the flagella (spermoids) occurs only in the blood extracted from the human body.

Fecundation can be artificially consummated under the eyes of the observer, but in nature it takes place only in the lumen of the stomach (middle intestine) of *Anopheles*. It is of fundamental importance to note that *Anopheles* sucking blood also ingest malarial parasites in various stages of development, but all those which are not mature gametes are digested. The mature gametes, on the other hand, survive and conjugate, giving rise to the formation of a zygote. This enters the wall of the mosquito's stomach and becomes encysted, forming a protrusion towards the

body-cavity. In a few days it increases to 80 μ in diameter, so that it can be readily seen with an ordinary lens.

Its development is completed in seven or eight days in a manner very analogous to what we have seen studying the cycle of Ross; that is to say, the nucleus of the parasite divides into numerous daughter nuclei, around which the protoplasm also divides, giving rise to the formation of an enormous number of fusiform sporozoites (probably not less than 10,000). At this point the capsule bursts and the sporozoites discharge themselves into the general body-cavity of the mosquitoes; from thence they reach the salivary glands by the lacunar circulation, and all collect there. The *Anopheles* when it bites a person injects saliva into the bite (probably to prevent the blood coagulating), and with the saliva the sporozoites are inoculated, which thus enter the blood, and begin and repeat in it the sexual generations of which we have already spoken. The researches of Grassi, Bastianelli, and Bignami have not been confined to the parasites of the æstivo-autumnal fevers, but have also included the hæmamœbæ of the tertian and quartan fevers, whose development they have also demonstrated in the clearest way in the body of the *Anopheles claviger*. At the commencement of development in the walls of the mosquito's stomach the parasites of tertian, quartan, and æstivo-autumnal fevers are readily distinguished from one another, especially by the character of the pigment, which is identical with that of the same parasites in man. Furthermore the zygotes of the quartan and also those of the tertian are distinguished by the nuclei being less numerous and larger, the sporozoites less crowded and arranged more regularly in rays, the residue of segmentation more numerous.

The agency of diffusion, then, of malarial infection is represented by the mosquito of the genus *Anopheles*, as Grassi has shown. The cycle is as follows: malarial man infects the *Anopheles*; the *Anopheles* in its turn infects man.

The malarial parasites have, therefore, two residences—the one with a constant and high temperature in the human body; the other with an inconstant and lower

temperature in the body of the *Anopheles*. Consequently we have to deal with a parasite having two alternating hosts—man and *Anopheles*.

In the *Anopheles'* body the parasite reaches a higher phase than that which is observed in man; in a certain sense it may be said, therefore, that man is the intermediate host and the mosquito the definitive host of the malarial parasites.

For the parasite to develop within the mosquito a certain temperature, however, is necessary; the æstivo-autumnal hæmamœbæ do not develop in the mosquito's body at a temperature below 15° C.; at 20° C. they develop slowly; at 30° C. they complete their evolution and produce sporozoites in about seven days.

This fact, discovered by Grassi, Bignami, and Bastianelli, has great epidemiological importance, inasmuch as it explains why malaria is not produced where the temperature of the environment is below 18° C.

The researches of Grassi tend to show that of all the species of mosquitoes which prevail in malarious places, those only which belong to the species of the genus *Anopheles* are the carriers of human malaria.

Grassi has observed the development of the malarial parasites in all the Italian *Anopheles*, which are—*Anopheles claviger*, Fabr., *Anopheles bifurcatus*, Linn., *Anopheles superpictus*, Grassi, *Anopheles pseudopictus*, Grassi.[1]

The Malaria Expedition, with Ross at its head, sent to Sierra Leone by the Liverpool School of Tropical Medicine, found the parasites of human malaria in two species belonging to that colony—*Anopheles costalis*, Loew, and *Anopheles funestus*, Giles.[2] And since the genus *Anopheles* is met with all over the world, it is probable that the species of this genus are everywhere the carriers of malaria. Guided by this idea, the entomologists of the British Museum are now studying the geographical distribution of *Anopheles*, and

[1] Grassi, "Le recente scoperte sulla malaria," 'Rivista di Scienze biologiche,' Luglio, 1899.

[2] Ross, "Malaria et Moustiques," Lecture faite à la Royal Institution of Great Britain, le 2 mars, 1900; 'Revue scientifique,' 4e série, tome xiii, 23 juin, 1900.

many hygienists, with Celli at their head, are studying with a practical criterion the mode of destroying the *Anopheles* and of preventing the spread of malaria.[1]

In *variola* some modern authors[2] have described Protozoa developing precisely within the cells of the stratum Malpighii of the cutis. These micro-organisms are reproduced by inoculating the variolous virus in the cornea of rabbits, where the parasites develop within the epithelial cells. Monti[3] has also met with these parasites in very small pieces of the skin removed with all necessary precautions from patients in the initial stage of hæmorrhagic smallpox (purpura variolosa). According to Monti, such researches have great diagnostic value in doubtful cases, where the patients die of hæmorrhagic smallpox in the initial stage of the disease.

In *molluscum contagiosum* likewise Protozoa have been constantly met with. They were well described and recognised as such by Perroncito[4] in 1882.

Nevertheless these discoveries have little diagnostic importance, inasmuch as the clinical characters of molluscum are very distinct, and the diagnosis is consequently very easy.

In *hydrophobia* up to the present micro-organisms have not been found; however, we owe to the genius of Pasteur precious methods for the diagnosis, prophylaxis, and treatment of this disease.

The diagnosis of rabies can be positively made when we procure the head of the biting animal.

[1] Celli, 'La malaria secondo le nuove ricerche,' seconda edizione, Roma, 1900. [This work has been translated into English by me, and published by Longmans, Green, and Co., London.—Translator.]

[2] Guarnieri, "Sulla eziologia della infezione vaccinica-vaiolosa," 'Archivio per le scienze mediche,' 1892.

[3] Monti, "I protozoi del vaiolo e del vaccino, e le localizzazioni del virus vaioloso nei colpiti da vaiolo emorragico," 'Atti dell' XI Congresso medico internaz.,' Roma, 1894, vol. ii, p. 128.

[4] Perroncito, 'I parassiti dell' uomo e degli animali utili,' Milano, Vallardi, 1882, pp. 93–4.

Admitting the fright which inspires the idea of the possibility of being attacked by this horrible disease, admitting also the new means by which we can prevent the explosion of hydrophobia in individuals bitten by rabid dogs, one readily understands the importance of having a positive method that enables us to establish whether the biting animal was truly suffering from lyssa or not.

For making the diagnosis the brain of the animal is extracted (always operating with the necessary precautions), and with a few drops of its emulsion a rabbit is inoculated.

The method preferred for this inoculation is the so-called subdural method of Pasteur.

A rabbit is fixed on the vivisection table; the cranium is trephined in the least vascularised region (the vault beside the median line), and a few drops of an emulsion of the suspected nervous system, previously diluted with a 75 per cent. sterile sodium chloride solution, are introduced between the dura mater and the surface of the brain; the bone is replaced and the skin stitched, always employing antiseptic measures. If the animal be mad the rabbits die in sixteen to eighteen days, with characteristic symptoms; that is to say, with a paralysis which begins at the posterior extremities and then runs forwards, determining tremblings of the head, and finally complete paralysis and death.[1]

We have found that one can advantageously substitute for the inconvenient method of subdural injection a simple inoculation of the cornea of a rabbit, using only a lancet charged with the infective material.

When the brain of a suspected animal has to be sent to a specialist to make the diagnosis, it should be preserved in neutral glycerine. In case the suspected animal has been already buried, it even after a few days may serve as material for research if it has not undergone putrefaction.

As the incubation period of hydrophobia is on an average from forty to sixty days, so we always have the necessary time for verifying the diagnosis by means of the

[1] 'Annales de l'Institut Pasteur,' from 1887 onwards; cf. Bordoni-Uffreduzzi, 'La rabbia canina e la cura Pasteur,' Torino, 1889.

inoculation of animals, in order eventually to have recourse to the Pasteur treatment, of which we shall speak later on.

Antisepsis and Asepsis.

As we have already mentioned in another chapter, Agostino Bassi must be considered the real founder of the parasiticide treatments. He, in fact, starting from the idea that all the contagious diseases, including ulcers and many skin diseases, are due to parasites, proposed to treat such diseases "with the use of substances or agents capable of killing the parasites, the authors of the fell disease, without taking the life of the beings which contain them."

Among the antiseptics Bassi recommended particularly corrosive sublimate, which he employed dissolved in the proportion of a grain in every two ounces of water. Such solution Bassi used for treating not only diseases and wounds of skin, but also for making urethral injections with the scope of curing gonorrhœa.[1]

But Bassi has confided to writings too brief, too rare, and little diffused, the fruits of his able mind and of his long experience. Besides, he lived in an environment very restricted, and therefore little adapted to the development and the application of his discoveries.

Ignatius Philip Semmelweis, the Hungarian obstetrician, instead, in 1847 made known to the University of Vienna—that is to say, to a great scientific centre—the results of his investigations on the etiology of puerperal fever, and the methods discovered by him for preventing it; but even Semmelweis was not listened to, and it is only within the last few years that the merit of having established the scientific and practical bases of obstetrical antisepsis has been duly credited to him.[2]

Semmelweis, struck by the extraordinary mortality of the puerperæ in the first obstetric clinic of Vienna frequented

[1] Bassi, 'Dei parassiti generatori dei contagi e rispettivi rimedi,' Lodi, Wilmaut, 1851.

[2] See Hueppe, Ignaz Semmelweis, Festredi, 'Comptes rendus et mémoires du VIII Congrès d'hygiène,' Budapest, 1894.

by the students of medicine, in comparison with that observed in other sections not frequented by the students, but only by the midwives, after having studied the cause of this difference, came to the conclusion that puerperal fever was due to infection, and precisely to the contamination of the uterus by the cadaveric virus transported by the students and the physicians who were in daily contact with the cadavers.

He then introduced into the clinic the methodical disinfection of the hands with a solution of chloride of lime before proceeding to the examination of the genitals of all the gravidæ and puerperæ. Immediately after the introduction of this rule the mortality fell from 10 per cent. to 3 per cent. But in November, 1847, having observed the appearance of the infection in twelve puerperæ following the examination of a woman who had an ulcerating cancer of the uterus, having also observed the transmission of the infection to diverse puerperæ from a woman who suffered from a gangrenous ulcer of the leg, he came to the conclusion that not only the cadaveric virus, but also the purulent or gangrenous exudates coming from living persons, were the vehicle of infection. Owing to this Semmelweis renewed the disinfection of the hands after each examination, introduced the use of the nail-brush to complete the disinfection, and the isolation of the infected patients.

Notwithstanding the successes obtained, communicated by Hebra [1] to the Society of Viennese Physicians in various lectures during 1847–8, notwithstanding the confirmation and approval of Professor Michaelis, of Kiel, Semmelweis was not listened to.

Even after he had published his great work [2] on the 'Etiology and Prophylaxis of Puerperal Fever,' the fruit of patient investigations, of able observations, and of experiences full of practical results, he encountered the opposition and the personal enmity of almost all contemporary obstetricians. The value of his great discovery was not

[1] Hebra, "Zeitschrift der k. k. Gesellschaft der Aerzte zu Wien,' vierter Jahrgang, 1847–8, Bd. ii, Seiten 242 bis 244, and 1848–9, S. 64–5.

[2] Semmelweis, 'Die Aetiologie, der Begriff, und die Prophylaxis des Kindbettfiebers,' Pest, Wien, und Leipzig, Hartleben, 1861.

recognised till many years after his death, principally owing to Hegar's publication.[1]

Only after the impulse given by Pasteur to the general doctrine of the pathogenic microbes was the environment made adaptable for the development of antiseptic medicine, and were the directive ideas for the prophylaxis and treatment of surgical infective diseases able to germinate.

Nowadays all the world honours the name of Lord Lister[2] as he who, inspired by the results of Pasteur's experiments on fermentations, created that new method of medication which, suppressing almost every possibility of infection after capital operations, rendered the success of the audacious surgeons almost infallible, to whom there is not much that surgery has not yielded.

The first publications of Lister appeared in 1867; but they had been preceded by the studies and works of an able Italian, which we are here obliged to record.

In 1866, in the 'Annali Universali di Medicina' Bottini published a remarkable work on the use of carbolic acid in practical surgery and in taxidermy.

In that remarkable work Bottini among other things wrote, "My investigations extended to more than six hundred patients, watching with the greatest diligence the changes occurring in ulcers dressed with carbolic acid, comparing this medication with others furnished by remedies of the same nature, suspending purposely the carbolic medications to discover how much was to be attributed to the action of this poison; finally, not admitting any treatment, to be able with full knowledge of the cause to deduce those corollaries which are the fruit of the pure and simple observation of the facts."

"In the tests made on anatomical preservations, having

[1] Alfred Hegar, 'Ignaz Philip Semmelweis, sein Leben und seine Lehre, zugleich ein Beitrag zur Lehre der fieberhafte Wundkrankheiten; mit einer Abbildung in Lichtdruck,' Freiburg-i.-B. und Tubingen, Mohr, 1882.

[2] See Schimmelbusch, 'Aseptische Wundbehandlung,' Berlin, Hirschwald, 1893; see also Löffler, 'Geschichtliche Entwickelung der Lehre von den Bacterien,' Leipzig, Vogel, 1887.

abundant material I undertook experiments on a vast scale, frequently testing identical organs preserved in carbolic acid and in other preservative liquids already known, in order to institute comparisons that would prove which was the best preservative."

Bottini employed a 5 per cent. solution of carbolic acid, thoroughly washing the part to be purified as well as wounds and suppurating sores with the solution; he also adopted carbolic acid "for internal use" in the treatment of cystitis, washing out the bladder with weaker carbolic acid solutions to destroy the vegetable and animal parasites, because "the complex facts that distinguish the phenomenon of cystitis may be promoted by the presence in the bladder of numerous parasitic tribes, which either owing to their vital acts, or to the chemical combinations that they determine in the urine, set up the process of inflammation." And treating of taxidermy he recommends the dissector disinfection with carbolic acid, because the latter, "arresting the putrid fermentation, destroys the virulent characters of anatomical wounds, the frequent cause of irreparable disasters."

In fine, to explain the action of the carbolic acid Bottini wrote:

"What is the mode of action of carbolic acid in arresting putrid decomposition?

"This is a question which is natural after having spoken of this antiseptic."

"If it be true that fermentation is the phenomenon produced by the action of a ferment on fermentescible matter, and that the ferment is a living organism, as Schulze and Schwann have held, and Pasteur with much ardour has recently supported, the explanation is easy, knowing in a positive way the morbific action of carbolic acid on microphytes and zoophytes."[1]

As is seen from the passages which we have cited, Bottini has preceded Lister[2] in the conception and in the

[1] Bottini, "Dell' acido ferrico nella chirurgia pratica e nella tassidermica," 'Annali universali di Medicina,' Dicembre, 1866, p. 585.

[2] Lister, "New Method of treating Compound Fractures, Abscesses, etc., with Observations on the Conditions of Suppuration," 'Lancet,' 1867; idem, "On the Antiseptic Principle in the Practice of Surgery," 'Lancet,' 1867, vol. ii.

practice of antisepsis. But Lister, with that practical spirit which forms one of the best characteristics of English genius, from the scientific discoveries of Pasteur deduced the general laws of antisepsis and the rules for their methodical application to practical surgery.

The fundamental idea of Lister was that of preventing infection; he recognised all the difficulties of the struggle against inflammation already manifested, all the dangers that the microbicidal substances in the concentration necessary for their effect would produce on the diseased organism. To prevent wounds being invaded by pathogenic germs he established the principle of rigorous disinfection, not only of the operative ground, but also of all the instruments and all the dressings.

We cannot here describe the original *technique* of Lister and all the improvements it has subsequently undergone. Of the Listerian antiseptic process both the materials of disinfection and the methods for applying them have now been modified; but the fundamental and marvellous conception remains intact, and has been confirmed and generalised as a highly humanitarian discovery.

Before the introduction of the antiseptic medication surgeons lost the greater number of their operation cases. Frequently the incision of a phimosis, the removal of a tumour of the scalp, the extirpation of an ingrowing toe-nail, etc., proved fatal.

Suppuration seemed necessary for the healing of certain wounds; erysipelas was domiciled in surgical wards; tetanus, septicæmia, and hospital gangrene often made their dreaded appearance in them.

The old surgeons used to say to their patients, "I have operated upon you; God will cure you." Luigi Porta, the celebrated Pavese surgeon, was so accustomed to see his patients succumb after operations ably performed that he considered them as material for his famous museum. Maisonneuve, discouraged by the frightful mortality of his operation cases, in spite of his readiness and consummate ability, during the last years of his life was obliged to abandon the knife and use caustics only.

Antisepsis has completely renovated surgery.

Operations which formerly produced terror, nowadays have become easy and perfectly safe.

Extirpation of the thyroid is performed without danger; hernia has ceased to be an almost incurable disease; furthermore, Bassini, after having operated on hernia, reconstitutes the inguinal canal.

Resections, osteotomies, enable limbs to be preserved which in other times surgeons would have amputated with little hope of success.

The suture of tendons, veins, arteries, has given sensational cures, thanks to the antiseptic method.

The older surgeons considered the peritoneum as a true *noli me tangere*, and, as from a fearful reef, tried to keep as far away from it as possible. A puncture, and perhaps even a simple scratch, made on the peritoneum was sufficient to make the wound fatal, whereas large incisions of the abdominal walls were considered of relatively little importance; hence the immense clinical and medico-legal differences between a penetrating and a non-penetrating wound of the abdomen.[1]

To-day the antiseptic method has rendered laparotomy so easy that this operation is performed with an exploratory scope to verify certain tumours of the belly. Lesions of the liver, spleen, kidney, intestine, and stomach have become accessible to the surgeon, and have been cured by opportune operative intervention.

In 1862 Sangalli, in his 'Clinical and Anatomical History of Tumours,' judged all attempts at ovariotomy unadvisable; owing to the progress of antiseptic surgery, Mangiagalli,[2] in 1895, was enabled to write his laparotomic work celebrating the triumphs obtained in several hundreds of ovariotomies.

The antiseptic method was rather slow in entering into obstetrics, and in Italy Mangiagalli was the first to call the attention of obstetricians to the value of this method in practical obstetrics.[3]

[1] Bottini, "Laparotomia antisettica," 'Studî sperimentali e cliniche reminiscenze del Corso 1878–9,' Milano, Dumolard, 1880.

[2] Mangiagalli, 'La mia opera laparotomica,' Milano, 1895.

[3] Mangiagalli, "Sul valore del metodo antisettico nella pratica ostetrica," ‛zetta medica Italiana,' 1878.

Now puerperal fever has disappeared from the obstetrical clinics, and it is destined completely to disappear when the prophylactic antiseptic precautions, nowadays recommended by all, formerly used only by Semmelweis, will be observed by all doctors and all midwives.

Even Cæsarean section is no longer an operation to be performed *in articulo mortis*, but, on the contrary, has become the ordinary way of delivery for women with a gravely deformed pelvis.

Microbiology and Hygiene.

The parasitic doctrine entering into hygiene has developed this science to such an extent as to have made it acquire fundamental importance in medical and sociological disciplines.

We should be obliged to go beyond our theme and write a large volume if we were here to summarise all the technical or hygienic measures for prophylaxis of the infective diseases. It is sufficient to say that from the microbiological doctrine precise rules have arisen for judging the purity of foods and drinks; for the sterilisation of infected materials, clothes, linen, rooms, ships, etc.

Preventive Immunisations and Specific Treatments.

Serotherapy.

The great principle of preventive immunisations against infective diseases is derived from the scientific analysis of the brilliant discovery made by Jenner in 1798,—that is to say, of Vaccination.

It is not necessary for us to insist on the advantages which vaccination has produced in preventing smallpox; innumerable authors have already treated this argument; and in a recent work Bizzozero[1] has demonstrated how

[1] Bizzozero, "La vaccinazione ed i suoi oppositori," 'Riv. d' Igiene e Sanità,' pubbl. N. 1—4, 1897.

erroneous are the ideas of the anti-vaccinators, clearly proving, by means of very rich statistical data, the great diminution of the mortality from smallpox verified in all countries where vaccination is practised. He has shown how false is the idea that other diseases are inoculated with the inoculation of animal vaccine, that the practice of vaccination has nothing to do with the supposed decadence of race, it not being true that with the diminution of smallpox other infective diseases have developed in its place.

The sanitary history of Germany demonstrates, on the other hand, in an undeniable way that with good vaccination smallpox disappears.

The most common method of vaccination is that of animal *vaccine*. This is prepared with the artificial cultivation from calf to calf of the original vaccine virus (cow-pox). For the inoculation and the collection of the virus male calves of from two to four months are preferred.

The animal is bound on the operation table, thoroughly cleansed and disinfected with boiled water. The hairs are shaved in all the lower abdominal region of the animal (including the scrotum), the parts are washed with warm sterile water, and dried with a sterile cloth. The skin is then as white and fine as that of a baby, to use the expression of the specialists; and it is very suitable for inoculation. The inoculations are made with sterile instruments, and may be performed in four different modes:

(*a*) By subepidermic punctures with a lancet charged with the virus.

(*b*) By incisions of variable length including only the superficial stratum of the derma, into which the virus is introduced by means of an ivory point.

(*c*) By cross-scarifications, produced by making a series of incisions very near to one another, from above downwards and from right to left.

(*d*) By denudation (Eternod's method),—that is to say, scraping the skin with emery-paper on a surface of several square centimetres, so as to lay bare the stratum Malpighii almost to the papillæ.

These surfaces (as also that prepared by process *c*)

are inoculated, rubbing them well with the ivory points charged with the vaccine.

The inoculations are allowed to dry before the animal is liberated from the operation table.

The calf is then led to a stall, where he is tied in such a way that he cannot rub or lick the inoculated parts. In the following days the general conditions of the animal are attended to; on the fourth day the animal is examined on the operation table. The virus is collected from the fourth to the sixth day, partly with forceps, partly with a surgical spoon, after having thoroughly washed the parts with sterile water.

In the former case the scabs are removed with the forceps, and then with a metal spatula the lymph and the pulpy matter are collected. In the latter case the epidermoid tissue of a greyish-red colour, which constitutes the efflorescence, is scraped with a surgical spoon, the collected pulp is triturated in a sterile mortar, and a little very pure neutral glycerine is added. The virus thus collected is distributed in capillary tubes, or in squares of glass according to Lancy's system.[1]

The vaccination of man is very simple. The part is thoroughly washed, at first with soap and then with sterile water. With a sterile lancet (platinum lancets which can be heated in the flame are now recommended) four to eight scarifications are made in the skin not quite to the corium. To insure the penetration of the virus the epidermis corresponding to the lesion is rubbed gently with the lancet charged with the virus; the lesion is then covered with animal taffeta, sterile cotton wool, and bandaged.

After four days or so papules surrounded by a halo develop in man, which are transformed into vesicles towards the fifth or sixth day, and on the eighth become purulent. Later the involution of the pustule and the formation of the scab take place.

The experience of a century, made not on a few animals but on hundreds of millions of persons, has demonstrated

[1] See Valliard, 'Manuel pratique de vaccination animale,' Paris, 1886; and Violi, "Vaccinazione e revaccinazione," 'Atti dell' XI Congresso med. internaz.,' 1894, Roma.

that a good and repeated vaccination is the sole means by which a nation can positively and durably subdue smallpox.

In these latter years, following the experiments and applying the same methods that have given such good results for diphtheria, a serotherapy of smallpox has been tried. But up till now, notwithstanding the efforts of Landmann,[1] Elliot, Beumer and Peiper,[2] Rembold,[3] etc., the researches on the serotherapy of smallpox have not reached any practical result.

Antirabic vaccination and the specific treatment of hydrophobia (or better the curative immunisation of those bitten by rabid dogs) is another of the remarkable discoveries to which Pasteur arrived, applying with scientific criteria the principle discovered by Jenner for vaccine.

Pasteur, after having demonstrated that the rabic virus is found in the central nervous system of animals dead of rabies, verified that the virus is reinforced by passing it repeatedly through the organism of the rabbit, and reaches such a constant degree of virulence as to determine the death of rabbits inoculated under the dura mater no longer in sixteen to eighteen days, but in eight.

The spinal cords of the animals killed by the fixed virus, preserved suspended in suitable glass vessels, in which are placed some pieces of caustic potash, thus maintained at a temperature of about 22° C., slowly become dry, and gradually lose their virulence in fourteen days.

Pasteur found that if dogs were inoculated for the first time (under the skin) with an attenuated cord of fourteen days, and then progressively with cords of thirteen, twelve, eleven days till the cord of the first day, these dogs became absolutely immune against rabies.

[1] Landmann, "Finden sich Schutzstoffe in dem Blutserum von Individuen, welche Variola bezw. Vaccine überstanden haben?" 'Zeitschrift f. Hygiene,' 1894.

[2] Beumer and Peiper, "Zur Vaccine-Immunität," 'Berliner klinische Wochen.,' 1895.

[3] Rembold, "Versuche über den Nachweiss von Schutzstoffen in Blutserum bei Vaccine," 'Centralblatt f. Bakteriologie,' Bd. xx, 1896.

This result already represents an extraordinary advantage for medicine, and would make rabies disappear, if by a legislative provision the vaccination of all dogs were made compulsory.

But Pasteur himself has rendered his discovery of much greater value, demonstrating that it is capable of preventing the development of hydrophobia in persons bitten by rabid dogs.

Such curative immunisation is now practised on a large scale on bitten persons during the incubation period of the disease, before the symptoms of the disease make their appearance.

In man also injections of the dried cords, dissolved in water, are made and introduced under the skin of the flank or abdomen, using progressively cords from the fourteenth day of drying to those of the third day.

If the injections be made with the necessary precautions there are no abscesses or other ill-effects.

For the bites of rabid wolves, in which the virus seems much more active, as also for face-bites (which have a shorter period of incubation), Pasteur introduced the *intensive method*, which consists in injecting at intervals of a few hours the emulsion of various cords in a single day, so as to exhaust the whole series in a few days. Then another series is begun, to render the immunisation more efficacious.

The Pasteur treatment has given undeniable advantages; *à propos* it is sufficient to consult the innumerable statistics published from 1887 onwards.

To reply to some opponents Pasteur himself, in 1889, collected the statistics of 1077 cases treated with his method in consequence of their having been bitten by animals certainly rabid, and recognised as such, inoculating a portion of the central nervous system of the suspected animals in rabbits, as we have described in speaking of the diagnosis of rabies.

In these 1077 cases Pasteur had a mortality of only 1·39 per cent.

In Italy also the Pasteur treatment has been largely adopted in apposite institutes opened in Rome, Turin, Milan, Bologna, Naples, and Palermo.

From the statistics published in Italy[1] it also results that the mortality from hydrophobia has extraordinarily diminished owing to the new treatment.

Tizzoni[2] within the last few years has tried a *serotherapy of hydrophobia,* vaccinating dogs and sheep with notable quantities of the nervous substance of rabid animals, attenuated in gastric juice.[3]

After eight to ten injections the serum of the animals acquires a notable vaccinating power, and a single injection of this serum is sufficient to render animals immune against the rabic virus.

According to the calculations of the author the injection of 20 c.c. of serum is sufficient for man even with a curative aim. This method is said to have the advantage of being very rapid and absolutely certain. Up till now, however, clinical experience has not proved that this method is of real value.

Diphtheria.—One of the most recent and greatest advantages derived by medicine from the doctrine of pathogenic microbes has undoubtedly been the principle of serotherapy. It has obtained splendid practical results, especially in the treatment of diphtheria. According to Behring, the principle of serotherapy is founded on a general conception developed from the systematic study of the pathogenic microbes and their actions on the cells of the organism. Behring's conception is as follows: the natural cure of diseases due to the specific virus is determined by the production in the organism of specific antitoxins. On the basis of this idea Behring and others have been stimulated to search for the mode of producing in the organisms of animals the specific antitoxins in such quantity

[1] Among the numerous statistics see Bordoni-Uffreduzzi, 'La rabbia canina e la cura Pasteur,' Torino, 1889; Germano e Calabrese, "Stastica e considerazioni sopra mille individui mossciati e trattati col metodo Pasteur," 'Giorn. intern. di scienze mediche,' 1894.

[2] Tizzoni, "Modo di preparare siero antirabbico ad alto potere curativo, e metodo di determinarne la potenza," 'Memoria della R. Accademia delle Scienze dell' Istituto di Bologna,' Serie V, 1895.

[3] Centanni, "Il metodo italiano di vaccinazione antirabbica," 'Riforma medica,' 1892.

and concentration as to be able to apply them with advantage in the treatment of human infections.

It is not our purpose to describe here the whole history of the serotherapy of diphtheria, which, besides, has already been done repeatedly by Behring[1] himself.

We shall speak briefly only of the new remedy and of the advantages derived from it.

The anti-diphtheritic serum is prepared in the following way:—Large cultures of virulent diphtheria bacilli are made in alkaline bouillon; after fifteen to twenty days these cultures are filtered through a Chamberland filter, and with the liquid collected sterile the immunisation of horses is begun. We commence by injecting under the skin 1 c.c. of the filtrate, and then on alternate days the inoculations are continued, always increasing the dose. The animals respond to the inoculations with a local and general reaction—œdema of the inoculated part, and rise of temperature. In increasing the dose of the toxin it is necessary to be very cautious not to endanger the life of the animal. After about a month a test bleeding is made, and the potency of the serum obtained is determined. To determine the value of an anti-diphtheritic serum the method suggested by Ehrlich, Kossel, and Wassermann[2] is now generally adopted. Such proceeding consists in mixing in a test-tube ten times the minimum lethal dose of toxin (the minimum lethal dose for a guinea-pig is in general ·1 c.c.) with various quantities of that serum whose potency we desire to test. The mixture is diluted with the sterile physiological solution, and injected under the skin of guinea-pigs weighing about 250 grammes.

Ehrlich has conventionally established to call that serum normal of which ·1 c.c. is sufficient to render innocuous ten times the minimum lethal dose of the toxin test. A toxin is called toxin test whose morbid action on animals is exactly determined, and the dose of which that produces the death

[1] Behring, 'Geschichte der Diphtherie mit Berüksichtigung der Immunitätslehre,' Leipzig, 1893.

[2] Ehrlich—Kossel—Wassermann, 'Deutsche medicinische Wochen.,' p. 16, 1894.

of a guinea-pig in the space of three to five days is precisely known.

The normal serum has one immunising unity per c.c.; and $\frac{1}{10}$ of a c.c. of this serum neutralises ten times the minimum lethal dose. A serum of which $\frac{1}{100}$ of a c.c. is sufficient to neutralise ten times the minimum lethal dose will possess, therefore, ten immunising units per c.c.; it will consequently be tenfold the normal serum. A serum of which $\frac{1}{1000}$ of a c.c. is sufficient to neutralise ten times the minimum lethal dose will possess 100 immunising units. According to Behring the anti-diphtheritic serum which is used for a therapeutic effect must not have less than sixty immunising units per c.c. In Italy, as a rule, the institutes which produce the anti-diphtheritic serum send out phials containing 8 to 10 c.c. of serum, a quantity which has 1000 immunising units.

The serum is derived by freely bleeding the horses with a special trocar, and receiving the blood in large sterile recipients (by means of tubes connected with the cannula) in such a way that the blood does not come in contact with the air. The blood is put aside in a cool place, then the serum is collected with particular expedients, and is distributed in phials.[1]

The injection of the serum in persons suffering from diphtheria is made with a large sterile hypodermic syringe, after having thoroughly washed and disinfected the skin. The injection may be made in any part of the body, but the interscapular region, the flank, or the skin of the abdomen is preferred, because in these parts the skin is less painful.

The effects of the injection of the anti-diphtheritic serum are truly wonderful. As a rule, one injection is sufficient,

[1] See Ehrlich and Wassermann, "Ueber die Gewinnung der Diphtherie-Antitoxine aus Blutserum und Milch immunisirter Thiere," 'Zeitschrift für Hygiene,' 1894; Behring, 'Das neue Diphtheriemittel,' Berlin, 1894; Monti, 'Osservazioni sulla preparazione del siero antidifterico,' Pavia, 1895, Soc. Med.; Brazzola, 'Preparazione del siero antidifterico,' Bologna, 1895, Accadem. Med.; Abba, "Il siero antidifterico," 'Gazz. med. di Torino,' Torino, 1895; Sclavo, "Relazione su alcuni sieri antidifterici," 'Riv. d' Igiene,' 1896.

because after twenty-four hours one observes detachment of the pseudo-membrane, lowering of the temperature, and diminution or disappearance of the glandular tumefactions. Only in a few cases is a second injection necessary.

If the treatment has been undertaken early, that is to say, in the first or second day of the disease, the cure is almost certain.[1] This is effected with greater difficulty when the treatment is employed later.

The advantages of the new method of treatment have nowadays been demonstrated by innumerable statistics collected in Europe, America, and even in Egypt, Asia Minor, and India. The materials gathered from the statistics are now so great that it is impossible to give here an analytical exposition of them. We shall observe only that before the introduction of the new method of treatment the medical statistics demonstrated that the mortality from diphtheria varied from 25 to 60 per cent. Baginski in the Children's Hospital of Berlin, for example, had always had an average mortality of 56—57 per cent. After the introduction of the new method of treatment he saw it descend to 16·23 per cent. In America Welch has collected universal statistics of 14,000 cases, and he has been able to determine that the mortality has fallen to about 14 per cent.

Concetti has collated the various statistics published by individual observers, dividing them according to the countries in which they were collected, and in a total of 17,429 cases he has observed that the mean mortality has declined to 14·51 per cent.

The curative property of the anti-diphtheritic serum is now recognised by the very large majority of practitioners. Some have indicated ill-effects which are observed after the injections, but such ill-effects are of little importance, and do not imply any danger in the use of the remedy. They are in general urticaria, erythema, and fleeting pains in the limbs. Some practitioners have imagined that the serum has an irritating action on the kidneys, but the majority of clinicians, on the other hand, have observed that the albuminuria is the effect of the diphtheria and not of the

[1] Baginski, 'Die Diphtherie,' Berlin, 1895.

remedy. In fact, the albuminuria exists before the injection and disappears after it.

Mya has injected large doses of the serum in healthy infants, and he has not observed any injurious effects from it.

Behring has recommended his serum also for preventing diphtheria; that is, he has suggested the injection of small doses of serum (200 immunising units) for rendering children refractory to the contagion.

Such preventive injections are now practised on a large scale in Germany and America; they have also been practised in many parts of Italy, and are highly recommended by Sormani, Belfanti, etc.

With regard to *tetanus*, Parietti,[1] of Pavia, was the first to demonstrate the possibility of a vaccination rendering animals immune against the tetanus infection by means of attenuated cultures.

Kitasato took up these experiments and performed vaccinations by means of the tetanus toxins obtained by filtration, weakened by the addition of terchloride of iodine. Behring obtained a similar result with cultures attenuated by terchloride of iodine or a solution of iodo-ioduret (Roux).

From the vaccinated animals Behring and Kitasato derived an antitoxic serum capable of preventing and curing tetanus. According to Tizzoni and Cattani[2] the tetanus antitoxin is a globulin, which, injected into the organism, renders those parts of the nervous system insensible to the poison that are not yet invaded by the disease. From horses Tizzoni and Cattani were able to obtain a serum which in their judgment possessed high immunising power. Vaillard, however, maintains that the anti-tetanus serum, while it has a curative effect on animals, has none on man.

Behring has now obtained the antitoxin in the dry state, which in doses of 5 grammes dissolved in sterile water,

[1] Parietti, 'Riforma medica,' 1889.

[2] Tizzoni e Cattani, "Sulla antitossina del tetano," 'Rif. med.,' 1894.

injected in horses, especially into the veins, has a curative effect.[1]

In man the injection is also made subcutaneously; the result, however, is favourable only when the injection is made within the first thirty-six hours of the disease. Up till now the clinic has not demonstrated sufficiently the efficacy of this remedy in the treatment of tetanus. Nevertheless preventive injections of the tetanus antitoxin are indicated in cases of suspected wounds.

In the treatment of *tuberculosis* the tuberculin of Koch has made us hope for grand results, and it has resulted in grave delusions.

Nowadays practice has demonstrated that tuberculin is only an eminently diagnostic means, and as such of great utility for recognising tuberculosis in animals, and thus preventing its diffusion from them to man.[2]

Maragliano, of Genoa, now prepares an anti-tubercular serum.

According to the author his serum has been proved innocuous, has had a depressing influence on the fever, has modified the local phenomena, has diminished or caused to disappear the bacilli in the sputa, has determined increase of body weight, has cured patients suffering from circumscribed apyretic tuberculosis, and has exercised a beneficial action on 91 per cent. of the cases. To these conclusions of Maragliano not a few objections and criticisms have been made; Foà, especially, has stated that the serum is not capable of curing animals affected by experimental tuberculosis. The clinic up till now has not pronounced a definite opinion on the value of Maragliano's serum.[3]

With regard to *glanders*, Nocard has extracted mallein from the cultures, which is used in veterinary practice for the

[1] Behring und Kolle, "Tetanus-Antitoxine," 'Deutsche med. Wochen.,' 1896.

[2] "Report of the French Commission," 'Bulletin de l'Académie de Méd.,' 1896.

[3] Maragliano, 'La sieroterapia nella tubercolosi,' Milano, Vallardi, 1896.

diagnosis of doubtful cases of glanders in horses. Some authors, however, such as Hermann, Schüyz, etc., even doubt the diagnostic value of mallein. Bonome[1] has tested mallein in the treatment of glanders in horses, and has had satisfactory results. Some trials of the sero-therapy of glanders also have been made, but up till now we have not sufficient experience *a propos*.

For *anthrax* of animals the preventive vaccinations introduced by Pasteur have been successful. He has placed in commerce two vaccines, a weak and a strong, attenuating the anthrax bacilli in cultures maintained respectively for twenty-four and twelve days at a temperature of 42° C.

Sclavo[2] has recently tried to prepare an anti-anthrax serum for combating the disease. The experiments of Sclavo are very promising; at present, however, we do not possess sufficient data to enable us to judge of the practical value of Sclavo's serum.

Just as I was correcting these proofs, at the meeting of the National Congress of Hygiene, held in Turin, November 1st, 1898, Sclavo gave the histories of the cases of malignant pustule cured up to then with the anti-anthrax serum.

They treated of fifteen individuals, to whom it is necessary to add two others, whose history was briefly reported at the close of the meeting by Dr. Pizzini.

For six cases of pustule observed in Buenos Ayres, the serum was prepared there by Drs. Mendez and Zenos.

Sclavo insists in stating that his serum so very quickly and profoundly modifies the course of the disease in man as to induce the conviction of the efficacy of the serum, and he discusses the importance of the new remedy, comparing it with the action of the remedies up till now recommended in practice.

The serum arrests immediately the spread of the local

[1] Bonome, "Sulla efficacia diagnostica e curativa dei prodotti del bacillo della morva," 'Rif. med.,' 1892, Congr. Med. inter., Roma, 1894.

[2] Sclavo, "Preparazione del siero anticarbonchioso," 'Gazz. medica di Torino,' 1895.

œdema, and determines its disappearance with great rapidity.

The reparative processes in the pustule are quickly initiated, so that the eschar soon falls off, and the subsequent cicatrix is very superficial and scarcely visible when the serotherapy has been adopted early.

In some persons also the rapid improvement of the general symptoms is surprising; the fever becomes quickly modified after the injection of the serum.

In the adynamic forms there is at first a rapid rise of temperature, even up to 41° C.; in those patients, on the contrary, in whom the vital forces are still strong, the serum causes the temperature to fall to normal in a short time.

A propos of this Sclavo reported some experiments made on sheep, which demonstrate that relatively weak doses of the serum, if they save the animal, are followed by the appearance of a high fever; while, when he injected endovenously rather strong doses of the serum, the temperature remained normal.

To save sheep 10 c.c. are sufficient when injected into the veins; advantages, therefore, may be expected from the serum also in veterinary practice.

The use of the serum never produced disturbances of any kind, though in a grave case Sclavo had injected 80 c.c.

For the treatment of malignant pustule in man Sclavo recommends the immediate injection of 20 to 30 c.c., repeating the dose after six to eight hours if the symptoms begin to mitigate.

Sclavo's serum does not lose its properties after two years of preservation.

After this new communication we must admit that Sclavo has truly given a new contribution, which constitutes a positive step forwards in the solution of a very difficult problem, namely, the therapy of anthrax.

In *purulent infections*, determined by streptococci and staphylococci, many authors have hoped to find a specific remedy in serotherapy. Viquerat has obtained an antistaphylococcic serum, vaccinating goats with broth cultures attenuated by chloride of iodine. Marmorek, Roger, and

others prepare a streptococcic antitoxin, vaccinating in diverse ways sheep, asses, and horses.

Notwithstanding this the clinical experiments made up till now by Petruschky, Baginski, Joiras, and many others give such very uncertain results that we cannot yet say whether the antitoxic serum is of any real advantage in the treatment of purulent infections or not.

In *pneumonia,* also, Foà in Italy, and Klemperer in Germany, have tried the serotherapic treatment; however, the clinical researches of Lava, Silva, and Righi have not demonstrated the efficacy of the serums up till now recommended.

For *typhoid fever* Pfeiffer and Kolle have proposed a method with which they hope to be able to protect healthy man from the infection during epidemics. The method consists in subcutaneously injecting small doses of the extinct cultures.

Owing to these injections the serum acquires the bactericidal and antitoxic properties which are observed in the blood of convalescents from typhoid fever. Nevertheless the real efficacy of this method of preventing the infection has not yet been demonstrated.

Chantemesse, Vidal, Beumer and Peiper have attempted to cure the developed typhoid infection with the serum of animals vaccinated strongly with cultures of the typhoid bacillus.

The clinical researches made up to now with this method are very scarce, and consequently a definite judgment on the value of the serotherapy is reserved to the future.

In *Asiatic cholera* numerous experiments on its prophylaxis and specific treatment have been made in these latter years.

In 1888 Gamaléia succeeded in rendering guinea-pigs immune against cholera, vaccinating them with attenuated cultures.

Haffkine has largely tested prophylactic vaccinations in man, subcutaneously injecting an attenuated culture, and

after five days a virulent culture of the cholera bacilli. With this method Haffkine, in India, has vaccinated 140,000 persons, and has obtained among the vaccinated an extraordinary diminution of the morbidity and of the mortality.[1]

The experiments on the serotherapy of cholera began after Lazarus had observed that the serum of those cured of cholera had an immunising effect on guinea-pigs. In the blood serum of vaccinated animals Pfeiffer, Isaeff, and Behring have demonstrated a substance which possesses vaccinating and curative properties. Ramson has isolated this substance from the serum, and has introduced it into medical practice. The necessary clinical experience, however, is wanting to judge of the curative efficacy of this substance.

Haffkine is continuing in India his experiments on the vaccination and the specific therapy of cholera, combining the injections of the serum with transfusion of sodium chloride solutions.

According to the observations of Kolle[2] the development of the cholera antitoxin reaches its maximum eighteen days after the inoculation; the effects of the anti-cholera vaccination last for a year.

These results agree with the practical ones obtained in India by Haffkine, who observed that the vaccination is complete on the twentieth day, and is maintained for a year.

In *bubonic pest* Yersin, Calmette, Borrel, etc., have tried vaccinations by means of broth cultures of the micro-organisms attenuated at a temperature of 58° C.

Yersin, immunising animals with large doses of attenuated cultures or with small doses of virulent cultures injected into the veins, obtained a serum capable of curing plague.[3]

[1] Haffkine, 'British Med. Journal,' Sept. and Dec., 1895; id., 'Münchener med. Wochenschrift,' 1895, etc.

[2] Kolle, "Experimentelle Untersuch. zur Frage der Schutzimpfung des Menschen gegen Cholera Asiatica," 'Deut. med. Wochen.,' 1897, No. 1.

[3] See the numerous notes published by Yersin and his collaborateurs in the 'Annales Pasteur' from 1894 onwards.

In a late number of the 'Annales Pasteur' Yersin refers to the successes obtained by him in India with the new curative method. The serotherapy of pest, consequently, appears very promising. Many authors are working on the subject, and in Italy Lustig announces that he has prepared an innocuous vaccine, deprived of bacteria, and a serum of extraordinary efficacy.

Thus, at the end of April, 1897, I finished my manuscript, presented to the *Concours* of the Cagnola Endowment, proclaimed by the Lombard Royal Institute, as has been said at the beginning of this book. Some very important publications, which have been issued between the closure of the *Concours* and the printing of these pages, oblige me to make some additions relative to the serum-therapy of bubonic pest and of yellow fever, without which my modest work would be too incomplete.

The specific agent of plague having been discovered, and Yersin having made known his first experiments on the immunisation of animals against pest, Lustig, with the collaboration of his assistant, Galeotti, undertook, in December, 1896, the search for a certain and rapid method of preventive vaccination. After having attempted in vain various methods, these authors obtained chemically from virulent plague bacilli of recent cultivation a toxic substance having the characters of a nucleo-proteid. It is not necessary here to refer at length to the *technique* for the preparation of this substance; we shall say only that masses of the bacilli are treated with a 1 per cent. solution of caustic potash, in which they become in great part dissolved. From this solution a precipitate is then derived by the addition of a small quantity of acetic acid. The precipitate, collected, washed, dissolved anew, filtered, and precipitated anew repeatedly, furnishes a substance completely free from living and dead bacteria, that, injected in small doses under the skin of sensitive animals, confers on them, after some days, an active immunity of long duration. Rats, which readily suffer from pest by natural

infection, once immunised become resistent, even after two months, to the intra-peritoneal inoculation of large quantities of the most virulent plague bacilli.[1]

This vaccine of Lustig, which, as is understood, has nothing to do with Yersin's vaccine, according to the studies of its discoverer, is almost innocuous for man when it is injected in single doses of two milligrammes. The injections cause only slight local and general phenomena for one or two days.

Lustig's vaccine, collected by the method above mentioned from recent and virulent cultures, keeps sterile and active for some months. In the dry state it does not lose its properties, remains sterile, and always offers the great advantage of permitting the dosage of the active substance for each inoculation.

If even other methods, using attenuated and virulent cultures, should be proved to confer active immunity, without producing any injury to the vaccinated, they would not concede the possibility of establishing precisely the quantity of the active vaccine used.

Continuing their experiments, Lustig and Galeotti found that animals highly immunised with repeated doses of the toxin after some time gave a serum endowed with distinct curative properties; and from horses vaccinated with strong doses they also obtained, after some weeks, a very efficacious anti-plague serum, sufficient in a dose of a cubic centimetre to cure a rat inoculated with a notable dose of very virulent plague bacilli.

To determine better the value of his discoveries Lustig went in person to India, where, exactly in Bombay, the plague raged. The English authorities, on account of the religious prejudices of the natives, did not think it prudent to permit a large experiment of vaccination on man. They allowed him, instead, to treat the patients with his serum. Then Lustig, considering that monkeys are very sensitive to plague, and that when they suffer from it they present a morbid picture similar to that of man, thought of testing his vaccine on the monkey. The numerous experiments made

[1] Lustig e Galeotti, 'Giornale della R. Accademia di Medicina di Torino,' March 26th, April 9th, and June 4th, 1897.

by Lustig and his assistants, Galeotti and Malenchini, aided by the assistants of the Parel Veterinary College, demonstrated that Lustig's vaccine protected monkeys in an absolute way from pest, even from the most rapid and severe forms, while all the non-vaccinated monkeys, contemporaneously infected with an equal quantity of the plague material died. On monkeys Lustig also proved the efficacy of his serum, and he had such encouraging results that he was suddenly induced to try it in men suffering from plague.

Having to deal with a new remedy and with a specific action, Lustig took care, above all, to ensure by every means possible the exactness of the clinical diagnosis.

The diagnosis of plague with a typical course, when the epidemic is active, offers no difficulty to an expert. When buboes manifest themselves the bacteriological examination is unnecessary; it is useful, though sometimes uncertain, when the buboes are not visible, and in the septicæmic forms in general. If in these forms the examination of the blood (cultures) be negative the cultivation of the liquid extracted by puncture of the spleen, of the cutaneous pustules, and of other tissues may be very useful.

Lustig's serum was administered by preference to those cases considered most grave by the English doctors. The septicæmic forms with or without buboes are very grave; the bubonic form, with a constantly high temperature, cardiac weakness, frequent dicrotic pulse, delirium, profound apathy, frequent respiration without pulmonary phenomena, enlargement of the spleen, cold sweats, scarce albuminous urine, is grave.

"Thirty cases of pest," writes Lustig, "were treated with the serum; of these four died, at the latest, within forty-eight hours.

"Of the thirty patients twelve had buboes in the groins, four only in the axillæ, five various buboes in diverse regions, two in the occiput, two at the maxillary angle, one a diffuse periglandular infiltration in the right groin; four had not buboes.

"Two patients died with buboes and septicæmia, two with septicæmia without apparent buboes.

"In the very grave advanced cases 40 to 60 c.c. of the serum was injected in the flank in one dose; as a rule, this dose was divided, and administered in from twelve to thirty-six hours. The absorption was rapid. In no case were local morbid phenomena observed.

"The effects of the serum are lowering of the temperature, sometimes slight collapse, diminution of the local pains, the headache, and the delirium. In no case was suppuration of the buboes verified, nor the frequent secondary complications.

"The serum was also efficacious even when, with the puncture, the bacilli were verified (by means of cultures) in the internal organs (liver, spleen) or in other tissues. The serum is inefficacious when the bacilli are found in large numbers in the circulating blood (septicæmia), or in the advanced forms, with grave phenomena of intoxication.

"Forty c.c. of the serum injected in one dose into a healthy person, or into persons suffering from malaria or croupous pneumonia, had no action.

"These clinical observations, recorded up till now with the greatest brevity, demonstrate the undeniable efficacy of the serum in a disease which gave in Bombay a mortality of about 85 per cent."[1]

After these brilliant results Lustig would have wished to extend on a large scale the use of his serum, but, his funds being exhausted, he sorrowfully was obliged to return to Italy after two months' stay in India during the worst of the seasons.

Though Lustig has been very guarded in proclaiming the importance of his studies, and has awaited with the greatest reserve and the most severe dignity that time and experience would confirm the results of his work, its importance has not escaped the English Government, which, when the pest again became prevalent, entrusted to Lustig the task of preparing his serum in large quantities for testing it on a vast scale, and at Bombay a Serotherapic Institute was

[1] Lustig, "Risultati delle ricerche fatte in India sulla vaccinazione preventiva contra la peste bubonica, e sulla sieroterapia," 'Rendiconti del l' Accadem. dei Lincei,' vol. vi, 2° semestre, serie v, fas. 8°, ottobre, 1897.

recently founded for the preparation of Lustig's serum and vaccine—an institute directed by Dr. Galeotti.

While awaiting the larger experience that Galeotti is now making in India on Lustig's serum, we believe it our duty, in describing these studies, to respect the prudent reserve of the celebrated Florentine professor.

In June, 1897, Dr. Giuseppe Sanarelli,[1] of Siena, director of the Institute of Hygiene of Montevideo, announced in a lecture that he had discovered the micro-organism of *yellow fever*.

Sanarelli had studied thirteen cases of yellow fever, and in seven of them he found, together with streptococci, staphylococci, *Bacterium coli*, *protei*, etc., a micro-organism up till then not well known, to which he gave the name of *Bacillus icteroides*. We must at the same time observe that, in 1890, Surgeon-General Sternberg,[2] of the American army, had already described a bacillus very similar to that of Sanarelli's, and had said, "It is possible that this bacillus is connected with the etiology of yellow fever." Sternberg expressed a similar opinion later on in his chapter on "Yellow Fever," in the excellent treatise of Davidson.[3] Nevertheless the observation of Sternberg passed almost unobserved, so that Sanarelli's discovery has been considered by most as a new one.

The *Bacillus icteroides*, according to Sanarelli, has a rodlet form, with rounded ends; is generally united in pairs in the cultures, and in little clumps in the tissues; it is from 2 to 4 μ in length, and is, as a rule, twice longer than broad. It is very polymorphous. In ordinary gelatine plate cultures it forms roundish, transparent, granular colonies, which, during the first three or four days, present the appearance of leucocytes. Subsequently the granula-

[1] Sanarelli, "La febbre gialla," 'Conferenza tenuta nella Università di Montevideo il 10 di Giugno,' 1897, Montevideo, Lib. Artistica, 1897.

[2] Geo. M. Sternberg, 'Report on the Etiology and Prevention of Yellow Fever,' Washington, 1890.

[3] Davidson, 'Hygiene and Diseases of Warm Climates,' London, 1893. See also Geo. M. Sternberg, "Der Bacillus icteroides (Sanarelli)," und "Der Bacillus X (Sternberg)," two articles in 'Centralblatt für Bakteriologie,' 1898.

tion of the colonies continuously becomes more intense, and generally an opaque nucleus is delineated. In time the entire colonies also become opaque, but they never liquefy the gelatine. Stroke cultures on sloped solidified gelatine develop, forming brilliant, opaque little drops, similar to drops of milk.

In bouillon it develops slightly without ever forming either pellicles or flocculent deposits.

In solidified blood-serum it grows in an almost imperceptible manner.

The culture on glucose (agar-agar), different from that which is verified for the greater number of the pathogenic microbes known, represents for the *Bacillus icteroides* a diagnostic means of the first order.

Nevertheless the demonstration of this diagnostic means is efficacious only in determinate conditions.

When the colonies develop in the incubator they assume an aspect which does not differ from that of many other microbic species,—that is, they are roundish, grey, a little iridescent, transparent, with a smooth surface and regular margins.

If, instead of being developed in the incubator at a temperature of 37° C., they are left to grow at the room temperature of 20°—22° C. the colonies form milky drops, opaque, raised, and with mother-of-pearl reflexes; that is to say, entirely different from those developed in the incubator.

We can then utilise this different manner of development, first exposing the cultures for twelve to sixteen hours to the temperature of the incubator, then keeping them for another twelve to sixteen hours in that of the room.

After this time the colonies are formed of a central flat nucleus, transparent and bluish, and of a peripheral circle, prominent and opaque, whose *ensemble* presents the exact image of a seal of sealing-wax.

As this character, which for the present may be considered as specific, can be put opportunely in evidence, even in twenty-four hours only, so it serves to establish in a very rapid and certain manner the bacteriological diagnosis of the *Bacillus icteroides*.

Besides these morphological characters, that are sufficient to differentiate the yellow fever microbe from all those at present known, the *Bacillus icteroides* is endowed with some interesting biological properties.

It is a facultative anaërobe, does not resist Gram's stain, ferments insensibly lactose, more actively glucose and saccharose, but is not capable of coagulating milk; it strongly resists drying, dies in water at 60° C., is killed by the solar rays in seven hours, and lives for a long time in sea water.

Foà[1] has had occasion to study a culture of the *Bacillus icteroides* sent him by Sanarelli himself, and he has confirmed almost all the morphological and cultural properties described, excepting the *seal* formation of the culture, which, according to Sanarelli, is specific.

The *Bacillus icteroides,* says Sanarelli, is pathogenic for almost all the domestic mammifers. The small rodents, after the injection, die in a variable time with reactions little specific.

The reactive animal *par excellence* is the dog, in which, according to Sanarelli, the symptomatology and the specific lesions of yellow fever are reproduced by means of endovenous injections.

Also in monkeys, goats, and sheep, besides grave fatty degeneration of the liver, nephritis, anuria, uræmic intoxication, and mixed infection are observed. Cultures filtered or sterilised with ether have a very high toxic power for the ordinary experimental animals. According to Sanarelli the morbid picture of yellow fever, both in man and animals, is due to a prevailing toxic process, produced by the active substances fabricated by the *Bacillus icteroides,* substances to which, as a whole, Sanarelli gives the name of amarylligenous toxin.

The amaryllígenous intoxication of the dog, still according to Sanarelli, besides reproducing the symptomatology and the specific lesions of yellow fever, also determines renal insufficiency, and favours the appearance of secondary infections on the part of the streptococcus, the staphylo-

[1] Foà, "Sul bacillo itterode (Sanarelli)," Accad. di Med. di Torino, Febbraio, 1898.

coccus, the *coli* bacillus, thus completing the exact reproduction of the picture that is observed in yellow fever of man.

After having experimented on the cat, the goat, the ass, and the horse, Sanarelli made experiments and inoculations on five men, and in this connection he expresses himself as follows:

"The injection of the filtered cultures in relatively small doses reproduces in man typical yellow fever, accompanied by all its imposing anatomical and symptomatological *cortège*. The fever, congestion, hæmorrhages, vomiting, steatosis of the liver, cephalalgia, rachialgia, nephritis, anuria, uræmia, icterus, delirium, collapse,—in fine, all that complex of symptomatic elements, which in their combined appraisement constitute the indivisible basis for the diagnosis of yellow fever, we have seen developing themselves before our eyes, due to the potent influence of the amarylligenous poison fabricated in our artificial cultures."[1]

All the symptomatic phenomena, all the functional alterations, all the anatomical lesions of yellow fever are nothing but the consequence of an eminently steatogenous, emetic, and hæmolytic action of the toxic substances of the *Bacillus icteroides*.

The anatomical alterations described by Sanarelli in experimental animals were confirmed in great part in diverse notes by Foà,[2] who, besides, has observed interesting lesions of the medulla of the bones, of the mesenteric lymphatic ganglia, of the intestine, of the central nervous system, etc. The alterations of the central nervous system observed with Nissl's method were described by Cesaris-Demel,[3] and, according to this author, from a simple swelling, with discoloration and breaking up of the chromatic part, they may reach to the complete destruction of definite nerve elements.

[1] Sanarelli, 'Etiologia e Patogenesio della febbre gialla,' Torino, Rosenberg e Sellier, 1897 (p. 136).

[2] Foà, "Sul bacillo itteroide (Sanarelli), Accad. di Med. di Torino," Febbraio, 1898; idem, "Ulteriori osservazioni sul bacillo itteroide," 'Gazzetta medica di Torino,' xlix, No. 15, 1898.

[3] Cesaris-Demel, "Sulle lesioni del sistema nervoso prodotte del bacillo itteroide," 'Rech. Accad. di Med. di Torino,' Marzo, 1898.

Finally, Sanarelli, in his book already cited, announced in July, 1897, that he had obtained from animals immunised with the amarylligenous toxin a serum endowed with preventive and curative properties.

In a lecture given before the Medical Society of St. Paul in Brazil—a lecture which we have not been able to read in the original, but only in the full summaries published in many journals—Sanarelli[1] communicated the results obtained from the first applications of the serum fabricated by him.

In the complex of twenty-two cases treated there were six deaths, or 27·27 per cent. According to Sanarelli this serum is bactericidal and not antitoxic. According to Foà,[2] who has had occasion to experiment on the serum sent him by Sanarelli, on the contrary, the action of the serum is antitoxic not by the direct action that the serum might have on the toxin, but by its capability of rapidly stimulating the formation of the antitoxin in the tissues.

These new researches of Sanarelli, though limited to a small number of experiments, have obtained in Europe very great popularity, owing to the numerous announcements of the political journals. Furthermore, we may say that after the famous anti-tubercular lymph of Koch, to no other discovery in the field of medicine has so much public attention been attracted as to these studies of Dr. Sanarelli.

But, on the other hand, the jounalistic *réclame* has produced a certain diffidence on the part of the scientific world, which has not yet been able to forget the delusions demonstrated owing to the supposed discovery of the anti-tubercular lymph, and which were eclipsed after a year of premature triumphs.

On our part any judgment on the whole work of Sanarelli seems at present premature; certainly for the dignity

[1] Sanarelli, "Premières expériences sur l'emploi du sérum préventif et curatif de la fièvre jaune: Résumé d'une conférence," etc., 'Annales Pasteur,' 25 Maggio, 1898.

See also Foà, "Sulla sieroterapia della febbre gialla," 'Gazzetta medica di Torino,' No. 14, 1898.

[2] Foà, "Sul modo in cui agirebbe il siero antiamarillico di Sanarelli," 'Gazzetta medica di Torino,' No. 17, 1898.

of science it would have been better if these results had been surrounded by that prudent reserve, which renders the indispensable researches of counter-proof easier and more serene.

Be this as it may, for the honour of the Italian name we hope that, the ready enthusiasms and unjustified anticipations having disappeared, Sanarelli's researches will be confirmed by dispassionate scientific analysis and daily clinical experience.

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